Vol. 9 No. 2

PLANT PHYSIOLOGY

APRIL, 1934

NUTRITIONAL REQUIREMENTS OF THE ROOT-ROT FUNGUS, *PHYMATOTRICHUM OMNIVORUM*[1]

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(WITH SIX FIGURES)

Introduction

A part of the general program of study of the destructive root-rot disease caused by *Phymatotrichum omnivorum* (Shear) Duggar has been a consideration of the nutritive relations of the fungus. The phenomenally wide range of host plants on which the root-rot fungus is an aggressive parasite suggests that knowledge of its nutritive requirements may add to our general concept of the parasitism of fungi, as well as increase our fundamental knowledge about this particular organism.

Phymatotrichum omnivorum has been grown in artificial culture at various times since ATKINSON (**1**) first isolated the fungus in 1892. He grew the fungus on sterilized sweet potato, cotton roots, apple roots, and manure. Later DUGGAR (**3**), TAUBENHAUS and KILLOUGH (**17**), and KING and LOOMIS (**8, 9**) described growth of the fungus on a wide variety of sterilized roots, stems, seeds, and fruits, and on agars prepared from such natural materials. The present work has been carried on concurrently with studies on the growth of the fungus in plant juices (**6**), and phases of the present work have been summarized previously (**5**).

Methods

The general plan has been to compare the growth of a single strain of *Phymatotrichum omnivorum* in flask cultures on the various synthetic media. Series of cultures were inoculated at the same time and incubated side by side at the specified temperatures. The dry weight of fungus

[1] Published with the approval of the Director as Contribution no. 215, Technical Series, of the Texas Agricultural Experiment Station.

growth produced was determined for each culture in which there was perceptible growth by filtering out the nutrient solution and drying the fungus colony in a crucible in the oven.

The strain of the fungus used in all the experiments was our no. 24, isolated August 6, 1929, from a sclerotium from a field at the Temple, Texas, Substation. This strain was proved parasitic and capable of causing typical root rot of cotton plants by successful inoculation experiments after 2 months and again after 27 months in artificial culture. Strain 24 has been carried in culture now for more than 2 years and 9 months and has shown no evidence of attenuation.

The first experiment was started from a tube culture of the fungus; but later series were inoculated with small pieces of sclerotia produced on the synthetic culture media. Sclerotial masses taken for this purpose were those produced on the bare walls of the flasks, above the level of the substratum, presumably precluding possibility of carrying portions of the old substrata over to the new cultures; and cultures for an entire experiment were always seeded with bits of sclerotia cut from the mass produced in a single flask. In practice, small portions of the sclerotial mass were pulled with a needle from the side of a flask, transferred to a petri dish containing moist, sterile, filter paper and cut with a scalpel into small pieces which were transferred to the cultures to be inoculated.

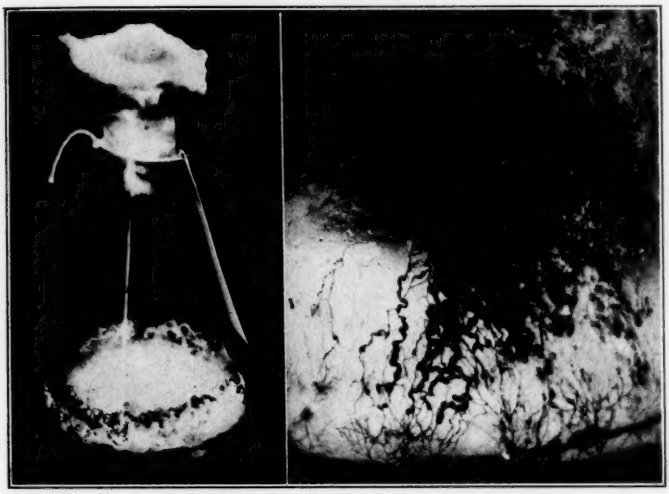
Chemicals used were of the chemically pure grade of Baker analyzed, Kahlbaum, or Difco products, but were not specially purified for this work. Unless otherwise specified, 50 cc. of the solution were used in each 250-cc. Erlenmeyer flask. Certain series included liquid nutrient solutions only; others included also parallel sets of agar slant or flask cultures, made up with 2 per cent. agar added to the respective solutions. The cultures were incubated on shelves in a large culture room, in which the temperature was held near 26°–28° C. during the winter by manipulation of the heating and ventilation, while in summer it rose to an average of 31°–33° C. The results within a given series are thus directly comparable but this is not true for results in different series.

The oven-dry weights of fungus growth were determined after the specified time by filtering the entire cultures through asbestos mats in Gooch crucibles in earlier work, and through alundum crucibles in the later series, washing rapidly with distilled water, and then drying in the oven at 90°–95° C. for 18 hours. The weights of colonies produced in replicate cultures were usually not very different, and only the average values have been tabulated. The reliability of these mean values is discussed in a later section.

Experiments

A preliminary experiment was started October 18, 1929, to find a liquid nutrient solution suitable as a starting point for the studies. Growth of

Phymatotrichum omnivorum was rapid and profuse in the artificial potato-dextrose medium devised by BROWN (**2**);[2] next in potato-dextrose decoction and agar; somewhat less in BROWN's simplified formula;[3] and none in SHIVE's "best" solution. Sclerotia developed profusely in anastomosing chains and matlike aggregations on the sides of the flasks in the complete BROWN's medium. This was the first record of production of sclerotia in pure cultures on synthetic media (fig. 1), although sclerotia had



A B

FIG. 1. *P. omnivorum* on synthetic media, showing masses of sclerotia: *A*, well developed sclerotial masses encircling flask culture 5 weeks old; *B*, sclerotia developing in chains and masses on wall of a 24-day-old culture.

been discovered some months earlier by KING and LOOMIS (**9**) in cultures on cotton and soil materials, and later found by NEAL (**12**) in the field. These sclerotia were used to inoculate succeeding series of flask cultures.

Later series were set up with various modifications of the complete BROWN's formula. In one series cultures were made with media which differed only by the omission respectively of one of the eight components of the complete formula. The fungus grew well in all the media except those from which starch or potassium phosphate had been omitted. In these two

[2] BROWN's complete medium (our solution no. 3) was made up to contain per liter (grams):—peptone 1.8, asparagin 1.8, dextrose 2.0, corn starch 40.0, $MgSO_4 \cdot 7H_2O$ 0.75, K_2PO_4 1.35, KCl 0.15, $FeCl_3$ trace.

[3] BROWN's simplified medium (our solution no. 4) was made up to contain per liter (grams):—asparagin 2, dextrose 2, $MgSO_4 \cdot 7H_2O$ 0.75, K_2PO_4 1.25.

media the early hyphal strands soon darkened and growth ceased, with no production of sclerotia; while with most of the other seven media, growth continued for several weeks and sclerotia appeared in many of the flasks.

Another series was used to compare growth of the fungus on media with constant nitrogen and mineral content but varying carbohydrate content. One solution included starch and a small amount of dextrose, as specified in the original BROWN formula; the other media included dextrose in amounts ranging from 2 to 40 gm. per liter. With the latter it was possible to filter off the substratum and to determine the weights of the colonies. Dextrose at 40 gm. per liter had supported the heaviest growth, averaging 106 mg. per flask after 16 days; while growth was less with lesser amounts of dextrose, and almost completely inhibited with only 2 gm. per liter or with none. Differences in the extent of growth corresponding to the differences in carbon content were observed also on agar slants made up from these nutrient solutions with the addition of agar (fig. 2). The growth

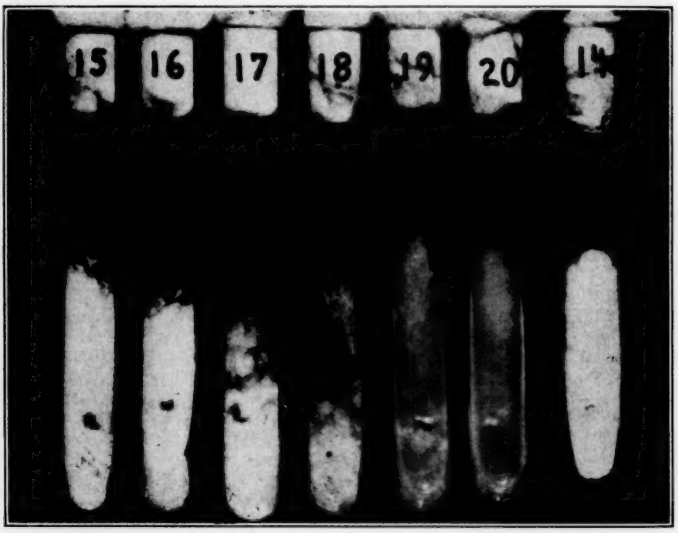


FIG. 2. Growth of *P. omnivorum* in 16-day-old cultures, on media with varying concentrations of dextrose (dextrose content decreasing from 40 gm. per liter in medium 15 to 20 gm., 10 gm., 5 gm., 2 gm., and finally to none in medium 20. Medium 14 contained 2 gm. of dextrose plus 40 gm. of starch).

obtained with dextrose was approximately as heavy as with the same concentrations of starch, and dextrose was adopted as the source of carbon for most of the later work.

Mineral nutrients

The effects of variation in the mineral content of solutions on the growth of the fungus are summarized in table I. The sources of nitrogen (peptone and asparagin) and of carbon (dextrose) were constant, while the mineral ingredients were varied in a number of combinations. The completed media were adjusted originally to approximately pH 7.0, using KOH or HCl respectively, except that H_2SO_4 was used with solution 54, NaOH with solution 57, and lime water with solution 58. The first four formulas differed by the cumulative addition to the basic formula of (1) magnesium sulphate, (2) potassium chloride, and (3) potassium phosphate. There was marked increase in growth only in solution 49, which included all three salts in what may be termed the "standard" concentration. Solutions 50, 51, and 52 included these three salts, with one of them at ten times the standard concentration. This increase in concentration encouraged slightly increased growth with magnesium sulphate, and somewhat reduced growth with potassium chloride and with potassium phosphate. In solution 53 the sulphate concentration was maintained at the same level as in solution 49, but magnesium was omitted and potassium increased, and markedly lighter growth resulted. This occurred also with the omission of the phosphate ion (solution 56), with substitution of sodium for potassium and magnesium (solution 57), and with substitution of calcium for potassium and magnesium (solution 58). It should be noted with regard to solution 58, however, that precipitation of tricalcium phosphate during neutralization of this medium had probably affected the concentration of phosphates available to the fungus. No significant change in growth resulted from omission of chlorine in solution 54, and but a small reduction in growth occurred in solution 55, in which the sulphate ion was omitted and the chlorine increased. Throughout the series, omission of potassium phosphate reduced growth more than omission of any other mineral constituent (fig. 3). Significant, though smaller, decreases resulted from omission of magnesium alone or of magnesium plus potassium.

The series summarized in table II included combinations of potassium and magnesium phosphates, sulphates, and chlorides, as well as a few combinations of sodium and calcium salts, as additions to a solution of ammonium nitrate and dextrose. Before autoclaving, media for this series were mostly adjusted to approximately pH 5.5 (instead of pH 7.0 as in most series), to allow for greater solubility of the magnesium salts; however, magnesium phosphate precipitates formed in solutions 97, 98, and 99. No adjustment was necessary for solutions 93, 97, 98, 100, and 101; sodium hydroxide was used for solution 103; ammonium hydroxide was used for solution 102; and phosphoric acid was used for the remaining solutions.

TABLE I

PRELIMINARY COMPARISON OF MINERAL NUTRIENTS AS AFFECTING GROWTH OF THE FUNGUS (INCUBATED AT 31°–35°, MEAN = 33.1° C.)

Nutrient solutions			Growth, solid and liquid cultures		Final results in 6 flasks of liquid after 5 weeks	
No.	Materials added to basic formula,* gm. per liter		Mycelium	Sclerotia	Final reaction of media	Mean dry weight of colonies
		gm.			*pH*	*mg.*
46	0 (basic formula only)		++	–	6.4	72
47	$MgSO_4 \cdot 7H_2O$	0.75	++	–	6.4	67
48	$MgSO_4 \cdot 7H_2O$	0.75	++	–	6.4	73
	KCl	0.15				
49	$MgSO_4 \cdot 7H_2O$	0.75	++++	++	5.2	463
	KCl	0.15				
	K_2PO_4	1.35				
50	$MgSO_4 \cdot 7H_2O$	7.5	++++	++	4.8	523
	KCl	0.15				
	K_2PO_4	1.35				
51	$MgSO_4 \cdot 7H_2O$	0.75	++++	+	5.5	445
	KCl	1.5				
	K_2PO_4	1.35				
52	$MgSO_4 \cdot 7H_2O$	0.75	+++	–	6.0	414
	KCl	0.15				
	K_2PO_4	13.5				
53	K_2SO_4	0.52	++++	Trace	5.3	170
	KCl	0.15				
	K_2PO_4	1.35				
54	$MgSO_4 \cdot 7H_2O$	0.75	++++	Trace	5.5	485
	K_2SO_4	0.175				
	K_2PO_4	1.35				
55	$MgCl_2 \cdot 6H_2O$	0.62	++++	+	6.3	408
	KCl	0.15				
	K_2PO_4	1.35				
56	$MgSO_4 \cdot 7H_2O$	0.75	+	–	6.1	77
	KCl	1.57				
57	Na_2SO_4	0.43	++++	+	5.3	101
	NaCl	0.117				
	$NaH_2PO_4 \cdot H_2O$	0.88				
58	$CaSO_4$	0.405	++	–	6.3	81
	CaCl	0.111				
	$Ca(H_2PO_4)_2 \cdot H_2O$	0.82				

* Basic formula 46 contains per liter (grams): peptone 1.8, asparagin 1.8, dextrose 40.0.

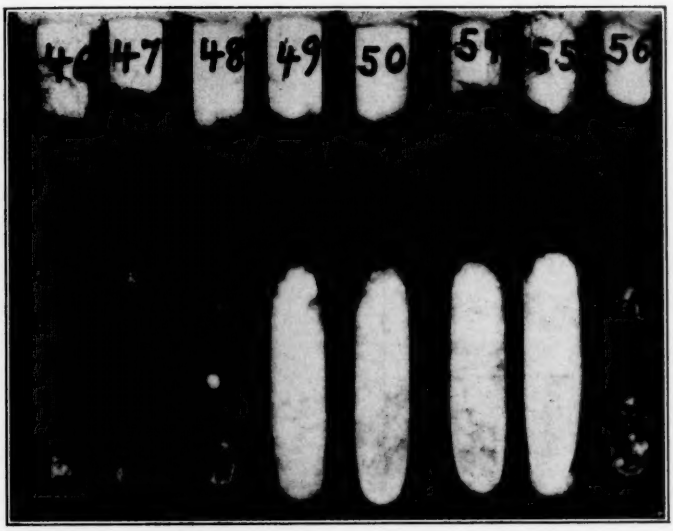


FIG. 3. Cultures of *P. omnivorum*, 15 days old, on media varying in mineral content. The importance of phosphate ion to growth of the fungus is shown by the poor growth in medium 48, which differed from 49 by omission of potassium phosphate, and medium 56 which differed from 49 by omission of phosphate ion and increase of chlorine ion.

The importance of the phosphate ion was shown again in this series. There was little growth in solutions 100 and 101, in which potassium and magnesium sulphates and chlorides respectively, were used to furnish the potassium and magnesium in the standard weights provided in potassium phosphate and magnesium sulphate of solution 104. Yet neither potassium phosphate (solutions 94, 95, 96) nor magnesium phosphate (solutions 97, 98) added individually produced appreciably better growth than in the basic solution alone (solution 93). A mixture to furnish the standard amounts of potassium and magnesium (solution 99) supported somewhat better growth, although still far inferior to that in complete formulas 104 and 70. A comparison of individual additions of different sources of phosphate, all to furnish the standard concentration of the phosphate ion, was furnished by solutions 94, 97, 102, and 103, which indicated that as the sole source of mineral nutrients, calcium phosphate was markedly superior to the potassium, magnesium, or sodium salt, as well as to the mixture of potassium and magnesium phosphate (solution 99).

The essential nature of the cations potassium, magnesium, or calcium was shown by the complete failure of growth in solution 105, which fur-

TABLE II

COMPARISON OF MINERAL NUTRIENTS, PARTICULARLY POTASSIUM AND MAGNESIUM, AS AFFECTING GROWTH OF THE FUNGUS (INCUBATED AT 24.5°–30.5°, MEAN = 27.9° C.)

NUTRIENT SOLUTIONS			INITIAL REACTION AFTER AUTOCLAVING	FINAL RESULTS AFTER 5 WEEKS		
No.	MATERIALS ADDED TO BASIC FORMULA,* GM. PER LITER			No. OF FLASKS	FINAL REACTION OF MEDIA	MEAN DRY WEIGHT OF COLONIES
		gm.	*pH*		*pH*	*mg.*
93	0 (basic formula only)		5.36	6	6.6	12
94	K_2HPO_4	1.35	5.35	4	4.8	14
95	K_2HPO_4	2.70	5.60	5	4.4	17
96	K_2HPO_4	6.75	5.50	6	5.2	3
97	$MgHPO_4 \cdot 3H_2O$	1.35	5.80	5	5.6	24
98	$MgHPO_4 \cdot 3H_2O$	2.70	6.15	6	6.2	16
99	K_2HPO_4	1.35	5.81	5	6.9	41
	$MgHPO_4 \cdot 3H_2O$	0.53	.....	.....	.....	.....
100	K_2SO_4	1.35	4.26	4	4.8	0
	$MgSO_4 \cdot 7H_2O$	0.75	.....	.....	.....	.....
101	KCl	1.15	4.92	3	4.2	7
	$MgCl_2 \cdot 6H_2O$	0.62	.....	.....	.....	.....
102	$Ca(H_2PO_4)_2 \cdot H_2O$	0.98	5.28	1	5.4	115
103	$NaH_2PO_4 \cdot H_2O$	1.07	5.39	2	5.2	5
104	K_2HPO_4	1.35	5.28	1	4.4	397
	$MgSO_4 \cdot 7H_2O$	0.75	.....	.....	.....	.....
70	K_2HPO_4	1.35	5.28	3	4.9	435
	$MgSO_4 \cdot 7H_2O$	0.75	.....	.....	.....	.....
	KCl	0.15	.....	.....	.....	.....
	$FeCl_3$	0.0015	.....	.....	.....	.....
105	Not added to basic formula but only:					
	$NH_4H_2PO_4$	0.89	5.56	3	4.4	0
	NH_4NO_3	0.87	.....	.....	.....	.....
	Dextrose	40.0	.....	.....	.....	.....

* Basic formula 93 contains per liter (grams): ammonium nitrate 1.18, dextrose 40.0.

nished the standard concentrations of phosphate, nitrogen, and dextrose, with all other materials omitted. Sulphur or the sulphate ion also is probably essential to good growth of the fungus. Solution 99 contained the same concentrations of ions as solution 104, except for omission of the sulphate ion and slight increase in the phosphate ion, but yielded only about

one-tenth as heavy growth as the latter solution. Growth was best with the standard mixtures of mineral nutrients provided in solutions 104 and 70. The difference between growth in these two solutions was scarcely sufficient to prove that iron and chlorine ions are involved in nutrition of the fungus.

Taken together, these results indicate the importance for the nutrition of the fungus of adequate supplies of most of the mineral ions of the original BROWN formula, including phosphate, potassium, magnesium, and probably sulphate. Calcium may possibly substitute for potassium and magnesium. Iron and chlorine, if needed, are supplied in sufficient quantities by impurities in the ingredients.

SOURCES OF NITROGEN

A preliminary series to compare different sources of nitrogen was set up in May, 1930, adding to an otherwise complete basic solution (solution 25, table III) a number of organic and inorganic materials in amounts calculated to furnish the nitrogen equivalent of 2 gm. and 20 gm. of asparagin per liter. The theoretical nitrogen content of the dilute solutions was 0.424 gm., and of the more concentrated solutions, 4.24 gm., of nitrogen per liter. The basic solution was adjusted to pH 7.0, but the individual solutions made up from it were not adjusted. Each solution was used in liquid cultures, and (with 2 per cent. agar added) in additional flasks and culture-tube slants. Ammonium nitrate supported the heaviest growth. The weights of growth obtained with ammonium nitrate, glycine, asparagin, urea, potassium nitrate, and leucine varied in the order mentioned. Growth of the fungus was less in the higher concentration of most materials.

The same sources of nitrogen were compared again in the cultures summarized in table IV. The plan of this experiment was similar to that of the earlier one, except that the higher rate of nitrogen supply used here was five instead of ten times the lower rate. The second series differed also in containing di-potassium instead of tri-potassium phosphate in the basic solution, and in the adjustment of all the completed solutions to approximately pH 7.0 before autoclaving. After autoclaving, the pH ranged from 6.2 to 6.52.

The heaviest growth was obtained again with ammonium nitrate at the lower concentration, followed by peptone, urea, glycine, asparagin, potassium nitrate, leucine, and ammonium sulphate in the order mentioned. There was heavier growth with the lower concentrations of asparagin, ammonium nitrate, and ammonium sulphate and heavier growth with the higher concentrations of peptone and potassium nitrate. In this series (table IV), as in the previous one, solutions with the lower concentrations of nitrogen generally became rather acid, while corresponding solutions with higher nitrogen contents generally became less acid or somewhat alka-

TABLE III

GROWTH OF *P. OMNIVORUM* WITH VARIOUS NITROGEN SOURCES, EACH TESTED IN AMOUNTS TO FURNISH 0.424 AND 4.24 GM. OF NITROGEN PER LITER. LIQUID CULTURES WITH 40 CC. OF MEDIA PER 200-CC. FLASK (INCUBATED AT 29°–35°, MEAN = 32.4° C.)

NUTRIENT SOLUTIONS			GROWTH IN AGAR FLASKS AND SLANTS	GROWTH IN SOLUTIONS AFTER 5 WEEKS		
No.	MATERIALS ADDED TO BASIC FORMULA,* GM. PER LITER			NO. OF FLASKS WITH GROWTH	FINAL REACTION OF MEDIA	MEAN DRY WEIGHT OF COLONIES
		gm.			*pH*	*mg.*
25	0 (basic formula only)		Trace	.....†	.....	.....
26	Asparagin	2.0	+++	.....†	.....	.....
27	Asparagin	20.0	+++	1	8.2	286
28	Leucine	3.96	+++	0	.....	0
29	Leucine	39.6	+++	1	6.1	178
30	Glycine	2.27	++++	2	4.7	301
31	Glycine	22.7	++++	1	6.5	300
32	Peptone‡	2.65	+++	0	.....	0
33	Peptone	26.5	++++	.....†	.....	.....
34	Urea	0.91	++++	3	5.9	237
35	Urea	9.1	–	4	7.2	15
36	Ammonium nitrate	1.18	+++	3	4.5	380
37	Ammonium nitrate	11.8	++	2	5.9	115
38	Ammonium sulphate	2.00	Trace	3	3.4	32
39	Ammonium sulphate	20.0	Trace	0	.....	0
40	Potassium nitrate	3.05	+++	4	8.2	228
41	Potassium nitrate	30.5	++	5	6.9	44

* Basic formula 25 contains per liter (grams): dextrose 40, $MgSO_4 \cdot 7H_2O$ 0.75, K_2PO_4 1.35, KCl 0.15, $FeCl_3$ 0.0015.

† Cultures lost.

‡ Nitrogen content assumed as 16 per cent.

line. Selerotia developed in cultures with asparagin, urea, peptone, and ammonium nitrate.

It is of interest to compare these results with those obtained by other workers. NEAL, WESTER, and GUNN (**13**) considered ammonium salts specifically unfavorable, since they found that ammonium nitrate and sulphate, used as sources of nitrogen in DUGGAR'S solution (in amounts to furnish approximately 12.4 gm. of nitrogen per liter), restricted growth of the root-rot fungus while growth occurred with various other nitrogen sources. These results are apparently in contradiction with our results, which instead indicate ammonium nitrate as the best source of nitrogen for the fungus. It is to be noticed, however, that NEAL and his associates used

ammonium nitrate and other nitrogen sources at concentrations nearly three times as high as those which have been shown here (table III) to be too high for best growth of the fungus.

In general, *P. omnivorum* utilized nitrogen well from organic and inorganic sources. It developed with amino acids, peptone, urea, ammonium salts, or nitrates as the source of this element.

TABLE IV

SECOND COMPARISON OF NITROGEN SOURCES FOR *PHYMATOTRICHUM*; EACH SOURCE USED IN AMOUNTS TO FURNISH 0.424 GM. OF NITROGEN PER LITER, AND ALL BUT LEUCINE, GLYCINE, AND UREA USED ALSO TO FURNISH 2.12 GM. OF NITROGEN PER LITER (INCUBATED AT 27.5°–36°, MEAN = 32.6° C.)

Nutrient solutions			Growth in agar flasks		Growth in solutions after 37 days		
No.	Materials added to basic formula,* gm. per liter		Mycelium	Sclerotia	No. of flasks with growth	Final reaction of media	Mean dry weight of colonies
		gm.				*pH*	*mg.*
62	0 (basic formula only)		+	–	6	6.4	7
63	Asparagin	2	++	++++	5	5.0	391
64	Asparagin	10	++++	++	6	7.4	310
65	Leucine	3.96	++++	–	6	4.4	177
66	Glycine	2.27	++++	–	6	4.9	428
67	Urea	0.91	++++	+++	6	4.7	439
68	Peptone	2.65	++++	+	6	4.6	462
69	Peptone	13.25	++++	–	5	7.4	531
70	Ammonium nitrate	1.18	++++	+++	5	4.5	566
71	Ammonium nitrate	5.90	++++	+	6	6.9	430
72	Ammonium sulphate	2.0	++	–	6	3.2	61
73	Ammonium sulphate	10.0	+	–	6	3.9	19
74	Potassium nitrate	3.05	++++	–	3	6.8	340
75	Potassium nitrate	15.25	++++	–	2	7.4	358

* Basic formula 62 contains per liter (grams): dextrose 40, $MgSO_4 \cdot 7H_2O$ 0.75, K_2HPO_4 1.35, KCl 0.15, $FeCl_3$ 0.0015.

SOURCES OF CARBON

The results of one series with carbon sources are summarized in table V. All of the materials tested supported better growth than was obtained in the checks, solution 78. Heaviest growth in 34 days was secured with the

higher concentrations of dextrose, maltose, and xylose; and definitely less growth with mannose, mannitol, lactose, and sucrose. With each source, the pH of the substratum at the end of the experiment was much lower for the more concentrated solution, and usually definitely alkaline for the less concentrated solution. Sclerotia were produced in three of the series, including the two in which heaviest growth was obtained.

TABLE V

GROWTH OF *P. OMNIVORUM* IN MEDIA CONTAINING DIFFERENT SOURCES OF CARBON, IN AMOUNTS EQUIVALENT TO DEXTROSE AT 40 AND 5 GM. PER LITER RESPECTIVELY MEDIA ADJUSTED TO APPROXIMATELY pH 7.0 BEFORE AUTOCLAVING (INCUBATED AT 25°–31°, MEAN = 28.2° C.)

NUTRIENT SOLUTIONS			INITIAL REACTION AFTER AUTOCLAVING	SCLEROTIA PRODUCTION IN FLASKS	GROWTH AFTER 34 DAYS		
No.	MATERIALS ADDED TO BASIC FORMULA,* GM. PER LITER				NO. OF FLASKS WITH GROWTH	FINAL REACTION OF MEDIA	MEAN DRY WEIGHT OF COLONIES
		gm.	*pH*			*pH*	*mg.*
78	0 (basic formula only)		6.54	–	5	8.1	23
79	Xylose	40.0	6.51	–	4	4.8	404
80	Xylose	5.0	6.58	–	4	7.8	57
81	Dextrose	40.0	6.56	++	4	4.9	532
82	Dextrose	5.0	6.77	–	3	7.9	50
83	Mannose	40.0	6.68	++	5	5.6	289
84	Mannose	5.0	6.68	–	4	7.8	94
85	Maltose	39.0	6.69	++	5	4.6	514
86	Maltose	4.88	6.91	–	5	8.0	57
87	Sucrose	39.0	6.94	–	5	6.8	154
88	Sucrose	4.88	6.94	–	3	7.5	95
89	Lactose	39.0	6.79	–	1	6.2	180
90	Lactose	4.88	6.88	–	3	6.6	102
91	Mannitol	40.5	6.91	–	4	7.0	224
92	Mannitol	5.06	6.97	–	5	7.6	129

* Basic formula 78 contains per liter (grams): peptone 1.8, asparagin 1.8, $MgSO_4 \cdot 7H_2O$ 0.75, K_2HPO_4 1.35, KCl 0.15.

In the series mentioned (table V), the carbon sources had been added to a solution which contained some carbon in the peptone and asparagin which supplied the nitrogen. A second series (table VI) was set up with an inorganic source of nitrogen, ammonium nitrate, so that carbon was supplied only by the various additions. The low growth in the check, as compared with solution 78 of the previous experiment, indicates that in the

earlier experiment the fungus evidently benefited from the carbon content of the organic sources of nitrogen used, and the low growth with the lactose cultures suggests that part of the growth with this sugar before may have been derived from the same cause. The trihydric alcohol, glycerin, appeared to be of no value to the fungus, although in the previous series the hexahydric alcohol, mannitol, was apparently a fairly good source of carbon, at least in the presence of the small amount of carbon already provided.

TABLE VI

GROWTH OF *P. OMNIVORUM* IN MEDIA CONTAINING DIFFERENT SOURCES OF CARBON, IN AMOUNTS EQUIVALENT TO DEXTROSE AT 40 AND AT 5 GM. PER LITER RESPECTIVELY. MEDIA ADJUSTED TO APPROXIMATELY pH 7.0 BEFORE AUTOCLAVING (INCUBATED AT 24.5°–29.5°, MEAN = 27.6° C.)

No.	NUTRIENT SOLUTIONS: MATERIALS ADDED TO BASIC FORMULA,* GM. PER LITER		INITIAL REACTION AFTER AUTOCLAVING	GROWTH AFTER 34 DAYS: NO. OF FLASKS WITH GROWTH	FINAL REACTION OF MEDIA	MEAN DRY WEIGHT OF COLONIES
		gm.	*pH*		*pH*	*mg.*
106	0 (basic formula only)		6.69	6	6.5	3
107	Dextrose	40	6.97	6	4.1	537
108	Dextrose	5.0	7.06	5	7.0	72
109	Lactose	39.0	6.97	4	5.3	41
110	Lactose	4.88	7.01	6	6.0	31
111	Glycerin	40.8	6.73	6	6.5	3
112	Glycerin	5.1	6.79	6	6.6	2
113	Corn starch	40.0	6.76	4	5.0	510
114	Corn starch	5.0	6.81	9	6.0	76
115	Corn starch, hot-air treated	40.0	6.36	6	5.5	508
116	Corn starch, hot-air treated	5.0	6.53	9	5.8	15

* Basic formula 106 contains per liter (grams): ammonium nitrate 1.18, $MgSO_4 \cdot 7H_2O$ 0.75, K_2HPO_4 1.35.

Commercial corn starch was included in this series, in the original untreated form in solutions 113 and 114, and in solutions 115 and 116 after inactivating vitamin A by treatment in a current of air in layers 0.25 inch deep in the oven at 110° C. for 24 hours. Weights of colonies in the concentrated starch solutions, 113 and 115, were obtained after boiling for 30 minutes with 10 cc. of 0.5 N sulphuric acid, to hydrolyze the starch, prior to filtration; this treatment probably also affected the weight of the remaining fungus mats. There was no apparent difference between growth with

the untreated corn starch (solution 113) and that with the devitaminized corn starch (solution 115), each of which supported nearly as heavy colonies as did the dextrose (solution 107) which again appeared to be the best source of carbon. Later comparisons of untreated with devitaminized starches (table X) yielded somewhat different results.

It is evident from these results that the fungus can utilize carbon from starch, disaccharide and monosaccharide (pentose and hexose) sugars, and even from the polyhydric alcohol, mannitol.

Course of growth in media of high and low dextrose content

The course of growth of the fungus on some media of known different nutrient values has been studied, largely to determine the period most suitable for comparison of weights of colonies. An extensive series was set up, using solutions 15 and 18, which differed only by containing respectively 40 and 5 gm. of dextrose per liter.[4] The cultures were seeded the same day, incubated side by side on shelves in the incubator room at 26°–31° C. with a mean temperature of 27.8° C., and ten (or fewer when necessary) flasks of each set selected weekly at random and removed for weighing.

After 2 weeks, the colonies in both media consisted of fairly light growth with concentric rings alternately of hyaline, submerged mycelium and of white, floating mycelium. After 3 weeks the entire surfaces of the solution 15 (4 per cent. dextrose) colonies were filled in with abundant mycelial growth, portions of which had already developed a light buff color. With solution 18, however, the concentric-ring growth was unobscured by any further development and remained approximately the same during the rest of the experiment. At the end of the third week, the colonies in solution 18 averaged only 157 mg., and the weights of colonies decreased progressively thereafter. In solution 15, the colonies continued to increase in weight through the fifth week, reaching then the peak weight of 558 mg., after which the weights of these colonies decreased each succeeding week also.

The average weights of colonies harvested after the various periods of incubation are given graphically in figure 4. An idea of how closely representative such averages were of the entire groups of cultures harvested at these times may be obtained from the standard deviations and coefficients of variation of these averages. For solution 15, the standard deviations were 1.8, 21.7, 38.8, and 47.8 mg. respectively, for the first 4 weeks of the experiment, and fell thereafter progressively to 37.6, 20.9, 14.7, 4.4, and 5.6 mg. However, the coefficients of variation decreased steadily from the first: 46, 34, 14, 9, 7, 4, 3, 1, and 1 per cent. By the third week the colo-

[4] In addition to dextrose, solutions 15 and 18 contained per liter (grams): peptone 2, asparagin 2, KCl 0.15, $MgSO_4 \cdot 7H_2O$ 0.75, K_2PO_4 1.35, $FeCl_3$ 0.0015.

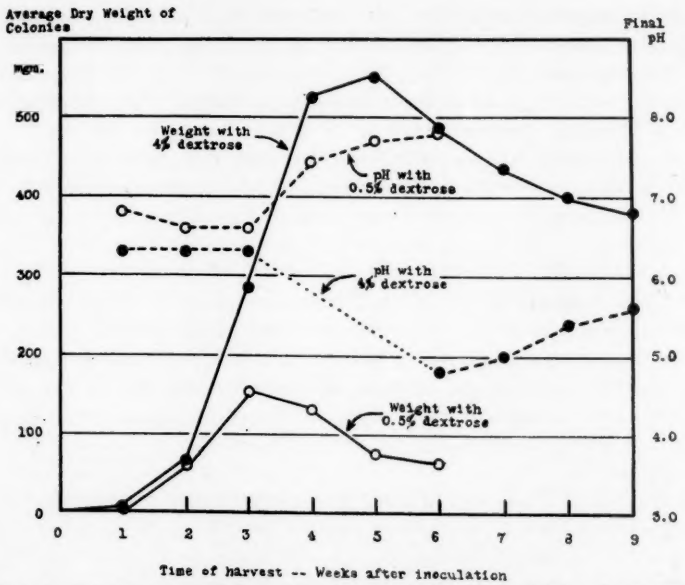


FIG. 4. Growth curves of *P. omnivorum* on solution 15, containing 4 per cent. dextrose, and on solution 18, containing 0.5 per cent. dextrose. Note rise in pH which occurred with cessation of increase in growth.

nies had grown to oven-dry weights of 230.3–357.5 mg., with the standard deviation of 38.8 mg. amounting to 14 per cent. of the mean weight of 286 mg. Although the colonies harvested 2 weeks later ranged from 496.8 to 618.6 mg. in weight, so many fell near the average weight that the standard deviation was only 37.6 mg. and the coefficient of variation only 7 per cent. In solution 18, the standard deviations decreased similarly after the third week, while the coefficients of variation decreased after the second week. With both media, the considerable variability of the younger colonies was thus quantitatively shown to be gradually minimized as growth continued, and the colonies were exposed to similarly limiting factors such as size of flasks, nutrient supply, temperature, etc.

With this information it would appear that, for a single representative harvest of the present series, a sample taken possibly at the end of the fifth week would yield more accurate results than an earlier sample. In such a single sample, on the other hand, growth might already have reached its peak and begun to decrease, as would be true here with solution 18. These considerations lead to two conclusions. First, in comparing weights of colonies in different media, as in tables I to VI, it is to be remembered that the

particular weights shown may represent samples from ascending, peak, or descending portions of the growth curves, and that these figures do not therefore represent specific indexes of the nutrient value of the various media, but merely indicate their probable relative value. Second, since samples after any particular period of incubation appear inadequate for careful comparison of substrata, harvests should be made when possible after 3, 4, and 5 weeks rather than after merely one period. This has been done with certain of the experiments to be discussed in a following section, and also in the study of plant juices as presented elsewhere (**6**).

Another growth experiment was run later, using three solutions with respectively 4, 2, and 0.5 per cent. of dextrose, and with ammonium nitrate as the source of nitrogen.[5] The cultures were incubated at 22°–32 C., with a mean temperature of 27.3° C. The weights of fungus colonies from five flasks of each solution, and of reducing sugars remaining in the culture solutions, were determined weekly, and are shown graphically in figure 5.

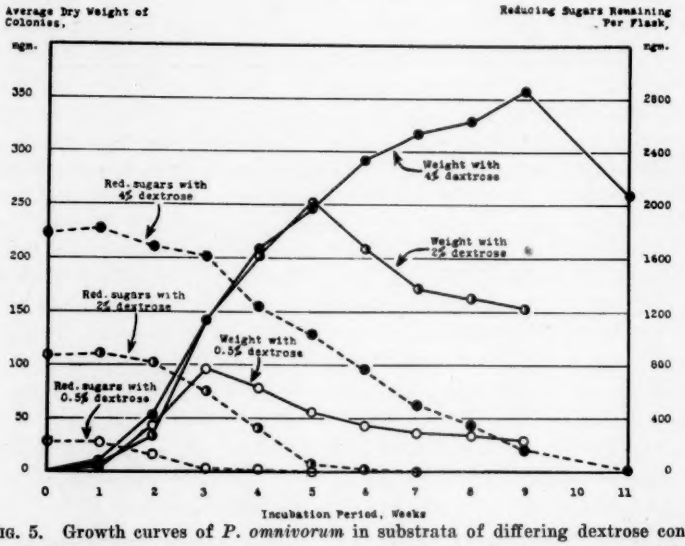


FIG. 5. Growth curves of *P. omnivorum* in substrata of differing dextrose content, showing weekly weights of fungus colonies, and weights of reducing sugars remaining in culture solutions. Note increases in colony weight until dextrose was almost completely exhausted.

In this second experiment growth was much slower than in the former series, the peak with 4 per cent. dextrose coming after 9 instead of after 5 weeks, and that with 2 per cent. dextrose after 5 instead of after 3 weeks.

[5] In addition to the dextrose, these solutions contained per liter (grams): ammonium nitrate 2.12, $MgSO_4 \cdot 7H_2O$ 0.75, K_2HPO_4 1.35, KCl 0.15, $FeCl_3$ 0.0015.

Colonies increased in weight in all three media at approximately the same rate, and apparently without regard to the differences in original concentration of dextrose; and this increase continued with each medium until most of the dextrose supply was exhausted. Thus with 2 per cent. dextrose solution, growth for 5 weeks was almost precisely parallel to that with 4 per cent. After 5 weeks, however, the dextrose supply had fallen to about 67 mg. per flask, and in the next harvest the fungus colonies showed a sharp decrease in weight. The supply of dextrose apparently became the limiting factor in growth of the fungus only after the dextrose concentration fell to around one-tenth of 1 per cent.

The decrease in weight of colonies in the descending portions of the curves must have been due in part to respiratory activity, and in part to autolytic decomposition of the older cells of the mycelium. This autolysis evidently did not destroy the viability of the cultures during the period of this experiment, since successful transfers to agar slants were made after 9 weeks from the cultures in the 2 and in the 0.5 per cent. dextrose solutions.

Taken together, the various studies on the relation of the root-rot fungus to different concentrations of dextrose illustrate the wide variations of conditions suitable for the fungus. It grew well in solutions containing about 16 per cent. of dextrose (table X), yet in media containing respectively 0.5, 2, and 4 per cent. of dextrose its rate of growth was constant until the dextrose content in the various solutions had fallen successively to a small fraction of 1 per cent. The fact that growth meanwhile continued in the media which contained the higher carbon contents indicated that further growth in the more dilute solutions was in fact prevented by the exhaustion of the carbohydrate rather than by the simultaneous increase in acidity. As shown graphically in figure 4, media became increasingly acid during active growth of the colonies. With exhaustion of the dextrose the colonies ceased to increase in weight, autolysis of the mycelium became predominant over growth, and the reaction of media shifted toward alkalinity. This relation was noted also in the second course of growth experiment as well as in other experiments. Media were generally more acid at the end of 3 or 4 weeks if the weights were at the maximum at that time, and more alkaline by the fifth week if the colonies meanwhile decreased in weight.

The explanation of this sequence is presumably the same as with most fungi. It is well established that fungi produce various organic acids, such acids as oxalic, citric, acetic, and kojic (**11**) having been identified as the products of fungus metabolism. The production of acid is generally linked with active growth in the presence of carbohydrates, and acids produced during growth may be utilized after the carbohydrate supply is exhausted. When this occurs, autolytic decomposition of the mycelium becomes the chief factor in further change of media, which become alkaline perhaps

mostly from evolution of ammonia. In alkaline media of this sort the basic radicals set free presumably combine with the carbon dioxide of respiration to produce bicarbonates, which have been thought by recent workers (**14**, **10**) to be the general cause of "staling" in old culture media.

Hydrogen-ion concentration of culture solutions

Earlier work with the root-rot fungus, particularly in its growth in soil and on cotton plants (**16, 4**), has shown that it is rather sensitive to acid reactions. In solutions acidified with hydrochloric, sulphuric, and acetic acids respectively, growth was inhibited by reactions of pH 4.1 and checked greatly in media more acid than about pH 6 (**16**). In the present studies, however, good growth was frequently obtained in media more acid than pH 5. This acidity was due to the presence of mono-potassium phosphate, of amino acids supplied as sources of nitrogen, or of acid products of the fungus metabolism. It has therefore been of interest to study the growth of the fungus in media made acid by additions of phosphoric acid.

In a preliminary series, cultures were grown for 5 weeks on media adjusted from pH 3.1 to 8.7 by additions of phosphoric acid and potassium

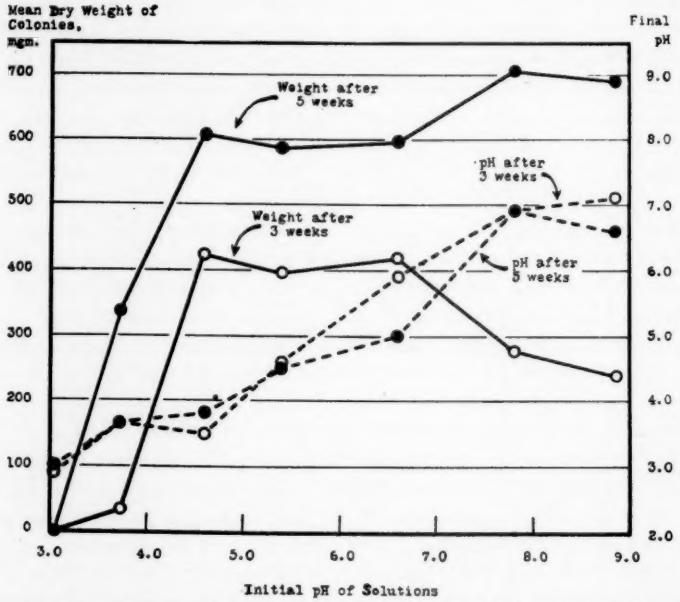


Fig. 6. Growth of *P. omnivorum* in solutions made acid by addition of phosphoric acid, and alkaline by addition of potassium hydroxide, respectively; and pH of media determined after initial aseptic adjusting and after respective periods of growth.

hydroxide respectively. Growth occurred in all except the most acid solution, which remained at about pH 3.0. Very slight growth, averaging only 41 mg., was obtained in the next most acid solution, which had been autoclaved after addition of the acid and had a final pH of 3.2. The other media, with initial values ranging from pH 4.1 to 8.7, and final values of pH 3.5 to 6.3, all supported good growth, the average weights of colonies in these media coming between 478 and 533 mg. Heaviest growth was in the two more alkaline substrata.

A more extensive series was set up later, adding (aseptically without further autoclaving) phosphoric acid for the more acid solutions, and potassium hydroxide for the more alkaline media, to nutrient solution 81 (table V). Five colonies in each solution were weighed after 3 weeks, and another five colonies after 5 weeks. The results are shown graphically in figure 6. Growth was inhibited in the most acid solution, at pH 3, but not in the most alkaline. Best growth was again in the two most alkaline solutions, which were adjusted originally to pH 7.81 and 8.85 respectively, but shifted approximately to neutrality by the end of 3 weeks.

These series indicate that the fungus may be somewhat less sensitive to phosphoric acid than to the acids used in the previous study (**16**). It is well known that the toxicity of acid culture solutions is influenced not alone by the hydrogen-ion concentration produced, but also by the specific acids present. Further direct comparison of the effect of different acids on the growth of *P. omnivorum* is planned.

Composition of fungus hyphae, and preliminary test for staling products

Observations on the root-rot fungus in culture solutions, in soil chambers, and on the roots of plants have shown that individual vegetative cells of the mycelium deteriorate comparatively rapidly as compared with the duration of the colony as a whole. Taubenhaus and Killough (**17**) described the breaking down of older cells of the hyphae and the characteristic production of deep brown droplets over the surfaces of colonies. In numerous inoculation experiments, it was found that the fungus did not remain viable on infected roots for many weeks after the inception of particular portions of lesions (**15**, p. 769–773). The question arose as to whether this deterioration of mycelium of the fungus under a wide variety of conditions, in some cases obviously from exhaustion of nutrients, might not be due partly to production by the fungus of materials toxic to its own further development, in other words, of "staling products."

A preliminary experiment was set up with 30 large, 1-liter flasks, each containing 200 cc. of culture solution 123 (table VII). Cultures were allowed to grow for 33 days; the solutions were then poured off and the colo-

TABLE VII

COMPOSITION OF *P. OMNIVORUM* MYCELIUM FROM 33-DAY-OLD COLONIES GROWN ON SOLUTION 123* AT 22°–32°, MEAN = 26.9° C. (ANALYSES ON TOTAL OF 13.8 GM. OVEN-DRY MYCELIUM)

	PERCENTAGE
Protein (N × 6.25)	29.25
Fat	4.02
Crude fiber	23.90
Nitrogen-free extract	35.62
Water	1.35
Ash	5.86
Ash included:	
Phosphoric acid (P_2O_5)	2.74
Potash (K_2O)	1.39
Lime (CaO)	0.12
Magnesia (MgO)	0.35
Insoluble ash	1.07

* Solution 123 contains per liter (grams): peptone 4.2, dextrose 40.0, $MgSO_4 \cdot 7H_2O$ 0.75, K_2HPO_4 1.35, KCl 0.15, $FeCl_3$ 0.0015.

nies dried for analysis. It may be noted (table VII) that the protein content of these 33-day-old colonies was higher than that of 21-day-old fungus mats grown in solution 81 and analyzed previously. With the latter, colonies with oven-dry weights from 331 to 426 mg. showed protein contents from 24.1 to 15.0 per cent. respectively, the higher percentages and higher actual amounts of protein coming in the lighter colonies.

The clear, reddish brown liquid poured off from the flasks still contained 3.98 gm. of dextrose per 200 cc., approximately half the original amount. This proportionate utilization of dextrose in 5 weeks compares well with that shown in the growth curves (fig. 5) for the same period, and suggests that the colonies in these large flasks were probably still growing actively.

Some of this old fluid was transferred with aseptic precautions to sterile flasks, and some to flasks containing previously sterilized, new synthetic culture solution. Duplicate series were run with autoclaved portions of the old fluid (table VIII). The fungus grew well in this old fluid alone, and mixtures of the fluid with the fresh solution made a nutrient medium which yielded heavier growth than the synthetic medium alone. These results therefore furnished no indication of formation of staling products by the root-rot fungus, at least up to a 33-day stage of growth under the conditions of this experiment. Inasmuch as many fungi are able to utilize the acids produced during their early development, and are said to be impeded only by too high concentrations of these acids or by the final alkaline

bicarbonates produced in autolytic decomposition, it will be of interest later to test the possible toxicity of culture media in which definite alkalinity has developed from autolytic decomposition of the fungus.

TABLE VIII

GROWTH OF *P. OMNIVORUM* ON OLD CULTURE LIQUID ON WHICH THE FUNGUS HAD PREVIOUSLY GROWN FOR 33 DAYS (SEE TABLE VII); 20 CC. OF MEDIA PER 150 CC. ERLENMEYER FLASKS (INCUBATED AT 23.5°–28.5°, MEAN = 26.0° C.)

COMPOSITION OF MEDIA	RESULTS AFTER 3 WEEKS			RESULTS AFTER 5 WEEKS		
	FINAL REACTION OF MEDIA	NO. OF FLASKS	MEAN DRY WEIGHT OF COLONIES	FINAL REACTION OF MEDIA	NO. OF FLASKS	MEAN DRY WEIGHT OF COLONIES
	pH		*mg.*	*pH*		*mg.*
Old fluid, aseptically removed and unheated, alone	6.4	1	76	5.6	2	118
Old fluid, autoclaved, alone	6.6	3	53	6.0	3	88
Unheated fluid, 5 cc. plus solution 70*, 15 cc	7.2	3	129	5.1	3	239
Autoclaved fluid, 5 cc. plus solution 70, 15 cc	7.4	3	116	5.2	3	248
Distilled water, 5 cc. plus solution 70, 15 cc	7.2	3	156	5.2	3	162

* For composition of solution 70, see table IV.

POSSIBLE ACCESSORY GROWTH-PROMOTING SUBSTANCES

In studies on growth of the root-rot fungus in plant juices (**6**), it has been found that addition to synthetic media of small amounts of expressed plant juices yielded rather large increases in fungus growth. This suggested that some accessory growth-promoting substances, perhaps similar to a vitamin, might be involved in nutrition of the fungus. Vitamins or similar substances have been found necessary to growth of certain other fungi. For instance, WILLAMAN (**18**) concluded that *Sclerotinia cinerea* (now *S. americana*) required an accessory material of properties similar to vitamin B. FARRIES and BELL (**7**) found that while many fungi grew on the simplified BROWN's medium utilized also in the present study (solution 4), *Nematospora coryli, N. gossypii,* and *Spermophthora gossypii* required in addition some active accessory material which was apparently present as an impurity in various natural protein sources. MARLOTH (**10**) has

TABLE IX

GROWTH OF *P. OMNIVORUM* IN SYNTHETIC MEDIA ALONE AND WITH ADDITION OF CARROT JUICE MEDIA ADJUSTED ORIGINALLY TO APPROXIMATELY pH 7.0 (INCUBATED AT 23°–32°, MEAN = 26.7° C.)

NUTRIENT SOLUTIONS*			INCUBATION PERIOD	MEAN DRY WEIGHT OF COLONIES, FROM SOLUTIONS WITH FOLLOWING ADDITIONS PER 50 CC.†	
No.	NITROGEN SOURCES	CARBON SOURCES		NONE (CHECK)	CARROT JUICE (2.5 CC.)
	gm.	*gm.*	*weeks*	*mg.*	*mg.*
68	Peptone 2.65	Dextrose 40	3	85	238
			4	217	361
			5	312	520
70	Ammonium nitrate 1.18	Dextrose 40	3	125	286
			4	235	562
			5	351	607
74	Potassium nitrate 3.05	Dextrose 40	3	71	230
			4	216	437
			5	211	530
81	Asparagin 1.8 and peptone 1.8	Dextrose 40	3	238	358
			4	498	662
			5	660	697
87	Asparagin 1.8 and peptone 1.8	Sucrose 39	3	345	387
			4	516	608
			5	715	780
124	Asparagin 1.8 and peptone 1.8	Dextrose 20 and sucrose 19.5	3	161	453
			4	457	644
			5	584	704

* For complete formulas of the solutions, see tables IV and V.

† Mean values for three flasks per series after 3 and 4 weeks, and for four flasks per series after 5 weeks.

noted that growth of *Penicillium digitatum* was so markedly accelerated by addition of a small amount of orange juice to synthetic media as to suggest that this fungus may require a vitamin occurring in citrus fruits for its growth.

Some experiments have been run to test the possibility of accessory materials being involved in nutrition of *P. omnivorum*. A first series was prepared from a uniform mineral base with the nitrogen and carbon sources varied as indicated in table IX. The same weights of carbon were

used in all, but the nitrogen content of solutions 68, 70, and 74 was only a little more than half that of the other three media. Each solution was used alone and also with the addition of 2.5 cc. of carrot juice (containing 0.17 gm. of dry material) per 50 cc. of solution (containing about 2.2 gm. of solid matter). The carrot juice was a portion of that prepared for other work (**6**, table VI) and had been expressed, autoclaved, filtered to remove the coagulum precipitated by the autoclaving, and re-autoclaved. Analyses showed that the 2.5 cc. of juice (1) added only 0.117 gm. of sugar to 2.0 gm. already present in 50 cc. of solution 68, for example; (2) added only 2.5 mg. of nitrogen to 21.2 mg. already provided; (3) added 6.0 mg. of potassium to 34.0 mg. provided; (4) added 0.5 mg. of phosphorus to 12.0 mg. provided; and (5) added 0.3 mg. of magnesium to 3.7 mg. provided.

Increases in growth were obtained with the carrot additions to all the solutions. The increases were markedly greater, both proportionately and absolutely, however, with solutions 68, 70, and 74 than with the other three solutions. The heavy growth in solutions 81, 87, and 124, even when used alone, was apparently due to the additional nitrogen and possibly to the particular sources of the nitrogen provided. This would suggest that increases following addition of carrot juice also may have been due to the nitrogen supplied in the juice. On the other hand, the asparagin used may have contained accessory growth-promoting substances as impurities and may have supplied these materials to the fungus.

The possibility that other ingredients of the formula might be carrying impurities influencing the results was considered in another experiment, in which ammonium nitrate was used as the source of nitrogen for all media. The growth obtained (table X) was similar with dextrose from an old lot and with dextrose from a new lot at 40 to 160 gm. per liter. Omission of ferric chloride and potassium chloride resulted in a slight decrease in growth, and a similar decrease occurred when all mineral ingredients were used at double strength. Accessory growth-promoting materials were therefore probably not present as impurities in the dextrose or mineral portions of the media; otherwise increasing the proportions of these ingredients should have resulted in greater accumulation of such impurities and greater growth.

The last two solutions of this series contained untreated and hot-air treated corn starch prepared in the same way as that tested earlier (table VI). In the present experiment both starches were dextrinized, prior to use in the culture solutions, by boiling 20-gm. lots for 150 minutes in 300 cc. water plus 25 cc. of 0.2 N hydrochloric acid, and then filtering. This hydrolysis did not transform much starch to sugar, and the larger amount of sugar was with the hot-air treated starch. Nevertheless more rapid and heavier growth occurred in the other solution. This agreed with other ob-

TABLE X

GROWTH OF *P. OMNIVORUM* IN SYNTHETIC MEDIA WITH VARIATIONS IN DEXTROSE AND MINERAL SUPPLY, AND WITH PLAIN AS COMPARED WITH HOT-AIR TREATED STARCH MEDIA ADJUSTED ORIGINALLY TO APPROXIMATELY pH 7.0 (INCUBATED AT 22°–30.5°, MEAN = 26.0° C.)

NUTRIENT SOLUTIONS		SUGARS IN 50 CC. OF AUTOCLAVED SOLUTIONS		MEAN DRY WEIGHT OF COLONIES†		
No.	MATERIALS ADDED TO BASIC FORMULA,* GM. PER LITER	TOTAL	REDUCING	3 WEEKS	4 WEEKS	5 WEEKS
	gm.	*gm.*	*gm.*	*mg.*	*mg.*	*mg.*
70	Dextrose 40	1.6864	1.6864	190	267	467
70B	Dextrose‡ 40	1.6864	1.6812	202	283	351
125	Dextrose 80	.....	.....	208	302	385
126	Dextrose 160	.....	.....	63	241	441
104	Dextrose 40 ($FeCl_3$ and KCl omitted)	.....	.....	156	237	316
129	Dextrose 40 (and all mineral ingredients doubled)	.....	.....	103	248	306
127	Corn starch, dextrinized, 40	0.1755	0.1715	249	419	578
128	Corn starch, hot-air treated then dextrinized, 40	0.3397	0.2842	43	57	348

* Basic formula contains per liter (grams): ammonium nitrate 1.18, $MgSO_4 \cdot 7H_2O$ 0.75, K_2HPO_4 1.35, KCl 0.15, $FeCl_3$ 0.0015.

† Mean values for three flasks per series after 3 and 4 weeks, and for four flasks per series after 5 weeks.

‡ Dextrose for 70B came from an old bottle of dextrose as used in previous experiments, the dextrose for all the other solutions being from a new lot.

servations with series prepared from unhydrolyzed starches, and thus not available for weighing. Heavier growth with corn starch that had not been given hot-air treatment (a difference not always obtained, see table VI) may have been due to the presence in untreated starch of vitamin A or some other material inactivated by the hot-air treatment, or to the presence in the hot-air treated starch of some product detrimental to growth of the fungus.

The uncertain nature of these results suggested a further test with known vitamin sources. Two general basic formulas were used (table XI), the first with solutions 63, 70, and 132, containing most of the ingredients in the standard concentration, while the formula used with solutions 130 and 131 contained much higher amounts of nitrogen and of minerals, to approximate the contents of complete carrot juice as shown by analysis.

Each basic formula was used with the various nitrogen sources indicated in table XI, and each complete solution was then used alone and with the addition respectively of three possible sources of vitamins. To one set of cultures, 2.5 cc. per flask of carrot juice were added, as in the earlier experiment (table IX); to another set, 1 drop (0.03 cc.) per flask of cod-liver oil[6] was added as a possible source of vitamin A; and to another series, 2 drops (0.087 cc.) per flask of rice-bran extract[7] as a possible source of vitamin B.

TABLE XI

GROWTH OF *P. OMNIVORUM* IN SYNTHETIC MEDIA ALONE, AND WITH ADDITIONS OF SMALL QUANTITIES OF VITAMIN SOURCES. MEDIA ADJUSTED ORIGINALLY TO APPROXIMATELY pH 7.0 (INCUBATED AT 29.5°–35.0°, MEAN = 32.9° C.)

NUTRIENT SOLUTIONS		INCUBATION PERIOD	MEAN DRY WEIGHT OF COLONIES, FROM SOLUTIONS WITH FOLLOWING ADDITIONS PER 50 CC.			
No.	MATERIALS ADDED TO BASIC FORMULA* † (GM. PER LITER)		NONE (CHECK)	CARROT JUICE (2.5 CC.)	COD-LIVER OIL (1 DROP)	RICE-BRAN EXTRACT (2 DROPS)
	gm.	*weeks*	*mg.*	*mg.*	*mg.*	*mg.*
63	Asparagin ..... 2.0 *	3	323	438	314	323
		4	498	629	522	492
		5	574	587	565	548
70	Ammonium nitrate 1.18*	3	244	429	347	280
		4	372	570	511	384
		5	503	529	589	512
132	Ammonium nitrate 1.18 and asparagin 2.0 *	3	365	586	490	483
		4	569	703	486	561
		5	570	560	583	513
130	Ammonium nitrate 1.41†	3	60	275	36	77
		4	87	464	44	258
		5	188	608	188	359
131	Ammonium nitrate 0.32 and asparagin 1.8†	3	25	114	19	39
		4	52	227	39	69
		5	81	307	81	103

* Basic formula for solutions 63, 70, and 132 contained per liter (grams): dextrose 40, $MgSO_4 \cdot 7H_2O$ 0.75, K_2HPO_4 1.35, KCl 0.15, $FeCl_3$ 0.0015.

† Basic formula for solutions 130 and 131 was calculated to contain with the nitrogen additions the approximate materials included in complete carrot juice and contained per liter (grams): dextrose 24.3, sucrose 22.4, $Ca(NO_3)_2 \cdot 4H_2O$ 0.93, KNO_3 2.95, K_2HPO_4 1.202, KCl 1.35, $MgSO_4 \cdot 7H_2O$ 1.16, $FeCl_3$ 0.0015.

[6] Cod-liver oil used was from a newly opened bottle from E. R. Squibb & Sons, and was labeled to contain not less than 70,000 U.S.P. units of vitamin A and not less than 2,600 units of vitamin D per 100 gm.

[7] Rice-bran extract prepared by soaking 10 gm. of commercial rice bran in 100 cc. of cold water for 2 hours, with occasional stirring, and then filtering to remove the bran.

All media were autoclaved after the various additions, so that thermolabile materials were presumably inactivated.

A question raised by the earlier results was whether asparagin might not have carried some accessory materials as impurities. In the present experiment, solution 63 contained as asparagin the same weight of nitrogen that was contained in solution 70 as ammonium nitrate. Growth was definitely more rapid in the former, and also attained a somewhat greater weight. On the other hand, solution 131 contained the same materials as 130 except that part of the nitrogen was supplied as asparagin instead of as ammonium nitrate, and it proved a poorer substratum for the fungus than solution 130.

The addition of cod-liver oil produced increased growth only with solution 70; with the rice-bran extract, growth increased definitely with solution 130, increased slightly in 131, and decreased in solutions 63 and 132. On the other hand, addition of carrot juice resulted in marked increases in the rate and final extent of growth with all five solutions. With the more concentrated synthetic bases, solutions 130 and 131, the addition of carrot juice appeared particularly valuable, apparently by obviating in some way some harmful effect from these concentrated media. In the concentrations tested here, vitamin A from cod-liver oil and vitamin B from rice-bran extract were apparently of little nutritive value to *Phymatotrichum omnivorum*, and probably neither of these materials is the basis for the increase in growth obtained from addition of carrot juice to synthetic media.

Synthetic media as influencing sclerotia production

For more than 2 years, series of 2 per cent. agar flask cultures have been maintained uninterruptedly to provide sclerotial material for use as inoculum in numerous lines of work. Each series included media prepared by two or three different formulas, and these were replicated periodically, usually to a total of 20–40 flask cultures of each substratum, until the more dependable medium for production of sclerotia was selected. An early series was made up from a basic formula similar to solution 48 (table I), except that corn starch was used instead of dextrose. Sclerotia developed profusely in 2 weeks in media to which mono-, di-, and tri-potassium phosphate respectively had been added at 1.35 gm. per liter, and which ranged in reaction from pH 4.6 to 8.4; but in a fourth series with no phosphate, sclerotia developed only after 4 weeks, and then in small quantities. The di-potassium phosphate medium (formula 44) was compared with media in which respectively the quantity of potassium phosphate was doubled, and dextrose was substituted for the starch; and much heavier sclerotia production appeared with formula 44. Formula 44, in which nitrogen was supplied partly as the relatively expensive asparagin, was then compared

with 76, in which all the nitrogen was supplied in 4.185 gm. of peptone per liter, and at least equally heavy production of sclerotia was obtained with the latter formula.

Formula 76 was found later to be much superior to media containing hot-air treated corn starch or dextrose instead of the untreated commercial corn starch. It was then compared with a more concentrated medium, 119, which included both dextrose and corn starch as sources of carbon, and both peptone and ammonium nitrate as sources of nitrogen. Formula 119 included the ingredients of solution 70 (table IV) plus 4.185 gm. of peptone and 40 gm. of dextrose per liter. In series repeated for more than 6 months, both media proved excellent for the production of sclerotia, but sclerotia developed almost invariably much earlier and frequently more profusely with 119.

Recent modifications include omission of the small amounts of KCl and $FeCl_3$, substitution of commercial sucrose for the dextrose, and rounding-off of the quantities of the other ingredients. The simplified formula 134[8] has now been tested for 10 months, in eight successive series. In all of seventy flask cultures with this substratum an abundance of sclerotia was produced, the masses extending around the flasks, reaching an average height of 30 mm. above the surface of the substratum, and often a thickness of 4–8 mm. This substratum, while doubtless susceptible of further improvement, can be recommended as useful for production of sclerotia. Omitting the corn starch produced a clearer material (formula 135), which has proved useful for general culture purposes with a number of other fungi previously carried on potato-dextrose agar, but has yielded only small quantities of *Phymatotrichum* sclerotia.

Comparative study of sclerotia production on the synthetic substrata just described, and also in the media included in previous sections of this paper, not only has made possible the development of dependable media for sclerotia production but also has furnished an insight into conditions which influence the inception of this stage of the life history, at least under these cultural conditions. It appears clear that nutritive conditions highly favorable for vegetative growth are also favorable for development of sclerotia. Nutritive and temperature conditions which impede vegetative growth apparently do not stimulate sclerotia formation, which is instead correlated with vigorous vegetative development.

Production of sclerotia in synthetic media in the laboratory is obviously not directly comparable with the development of sclerotia under natural conditions in the field. Yet it appears probable that production of sclerotia

[8] Formula 134, used in cultures for sclerotia production, includes per liter (grams): peptone 4.2, ammonium nitrate 1.2, corn starch (commercial) 40.0, sucrose (commercial) 40.0, $MgSO_4 \cdot 7H_2O$ 0.75, K_2HPO_4 1.5, agar 20.0.

in the field also is correlated with conditions of rapid growth and abundant nutrient supply for the root-rot fungus, rather than with conditions of retarded growth and insufficient nutrients. It would follow that treatments which militate against rapid and free vegetative growth of the fungus in the field might have a further desirable effect in discouraging production of these long-lived resting bodies of the fungus.

Discussion

From comparative studies of growth of *Phymatotrichum omnivorum* on numerous synthetic culture solutions, it is found that the nutrient requirements of the fungus are not very exacting. This accords with the wide range of host plants, including possibly the majority of gymnosperms and dicotyledonous plants, which are attacked by this fungus. It is well known, however, that many fungi with restricted host ranges can nevertheless be cultured on similarly varied synthetic media. Within the limits of the nutrient variables considered in the present work, therefore, the extent of nutrient conditions under which the root-rot fungus can be grown would appear neither particularly unusual nor likely to be of much significance in relation to the host range. This conclusion agrees with results obtained in the concurrent study of growth of the fungus in plant juices (**6**), which showed that immune monocotyledonous plants were protected, not by lack of necessary nutrient materials, but by the presence of some specifically toxic material.

Summary

1. Mineral ions utilized by the root-rot fungus, *Phymatotrichum omnivorum*, were phosphate, potassium, magnesium, and probably sulphate. Calcium, iron, and chlorine, if needed, were supplied in sufficient quantities by impurities in the ingredients.

2. Nitrogen was utilized equally well from organic sources, such as amino acids, peptone, and urea, and from inorganic ammonium and nitrate salts. Ammonium nitrate was frequently the best source of nitrogen.

3. As sources of carbon, the fungus utilized pentose and hexose monosaccharide sugars, disaccharide sugars, starch, and to a lesser extent the polyhydric alcohol mannitol.

4. The fungus grew at the same rate in media containing various concentrations of dextrose. Colonies increased in weight and the media became increasingly acid until the dextrose content of the solutions fell to a fraction of 1 per cent. After exhaustion of the dextrose, weight of colonies decreased and the reaction of the media changed rapidly toward alkalinity.

5. In media adjusted to various reactions by phosphoric acid and potassium hydroxide respectively, best growth was obtained in the more

alkaline solutions. Growth was inhibited at approximately pH 3, but good growth occurred at pH 3.7.

6. No indication of staling products was obtained in tests of substrata in which the fungus had grown for 33 days.

7. Small quantities of carrot juice added to synthetic media resulted in disproportionately large increases in growth of the fungus, and suggested that some accessory growth-promoting material might be involved. In one experiment, addition of carrot juice to solutions containing asparagin as a source of nitrogen resulted in smaller increases in growth than were obtained with carrot juice additions to other media. Addition of cod-liver oil (as a source of vitamin A) and of rice-bran extract (as a source of vitamin B) to culture solutions produced insignificant changes in growth of the fungus. The basis of increased growth with the juice addition may be the specific organic nitrogen source provided in the juice, some accessory growth-promoting material, or some other factor.

8. Sclerotia developed most abundantly in media most suited for rapid and abundant vegetative growth. The formula of a nutrient medium developed specifically for sclerotia production is given.

9. The variety of nutrient conditions suitable for growth of this fungus accords with the wide host range of the fungus. Limitation of hosts of root rot, however, has already been shown to be determined apparently by the presence of toxic materials in immune monocotyledonous plants, rather than by the lack of nutrients in such plants.

The writers take pleasure in expressing their appreciation of the advice of Dr. G. S. FRAPS, Chief of the Division of Chemistry, in connection with various phases of the work.

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EFFECTS OF HUMIDITY ON METABOLISM IN TOMATO AND APPLE[1]

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(WITH ONE FIGURE)

The literature on effects of specific factors of environment includes reports which are often contradictory in greater or less degree. The reason sometimes would seem apparent. In considering the results obtained with respect to plant appearance, anatomy, or composition, the causal factor has often been assumed by the investigator to be solely that which was varied experimentally. Perhaps the most that may be said is that when one or another factor of environment is apparently dominant, as temperature, for example, metabolism and growth of the plant may under that temperature and specific total-environment show certain trends; trends, however, which can be accentuated or masked by modification of nutrition or carbon dioxide supply, light intensity, or other factors (**22**).

The quality of growth of plants, however, is always the expression of the specific character of their metabolic processes involving all assimilated products (**15**), and in part unassimilated materials (**8, 12, 14, 16**), guided and often qualitatively and quantitatively directed by the factors of the environment. A shift in one or more of these factors is reflected in the type of growth of the plant. With these points in mind, a study was made of the growth responses and accompanying chemical changes which were found to take place in tomato plants subjected to an environment in which humidity was the single variable factor for a given nutrient treatment. The nitrate reducing power of these plants, together with microscopic observations, are reported in detail by ECKERSON (**4**), although frequent reference to her work will be made in the following pages.

PART I. TOMATO

Experimental methods

Recent papers (**11, 19, 20**) record the results of experiments in which plants were grown at the same relative humidity but at different temperatures, the lowest being 45° and the highest 95° F. The rate of evaporation from leaves must be closely related to the vapor pressure deficit of the

[1] Through the courtesy of the University of Chicago there was made available for these experiments the temperature-humidity control equipment of its Botany Department. The writers wish also to express appreciation of the cooperation of C. S. CATHCART in making Kjeldahl determinations and of G. B. ULVIN'S assistance in making chlorophyll analyses.

surrounding air. Figure 1 shows that although a constant relative humidity may be maintained, the vapor pressure deficit of air varies as the temperature changes. It was found practically impossible, in the experiments cited, to maintain the same vapor pressure deficit at the extremes of temperature employed, but relatively easy to maintain the same relative humidity. The significance of vapor pressure deficit in relation to phases of metabolism other than transpiration is problematical. Because of this fact and because of the practical impossibility of maintaining the same vapor pressure deficit under these extremes of temperature, it was empirically chosen to maintain the same relative humidity at all temperatures. This

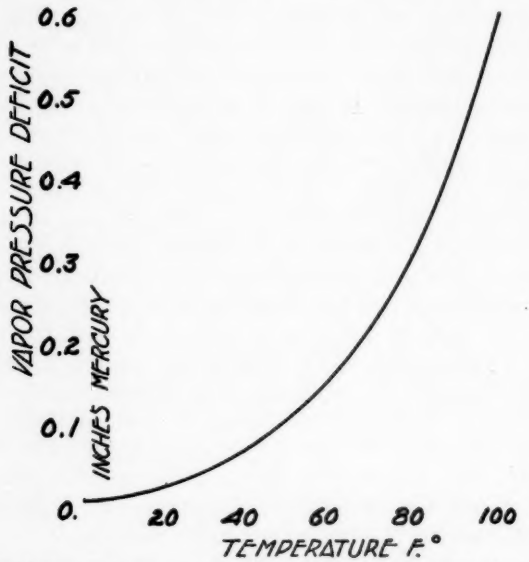

FIG. 1. Variation of vapor pressure deficit of atmosphere with rise of temperature at a maintained relative humidity of 70 per cent.

method of expression of the moisture content of the air is adhered to in this, as well as in the papers cited, and is commonly used in horticultural practice.

Tomato plants of the Bonny Best variety were employed for these experiments. They were grown for four weeks in sand culture with no external nitrogen supply, in new, washed, 10-inch clay pots, five plants to a pot, under prevailing greenhouse conditions. The pots were set in shallow enamelware pans which were kept constantly full of nutrient solution, which was applied daily in sufficient quantity completely to flush the sand.

On May 31, after the plants had been grown for four weeks with minus-N solution, some of them, later referred to as initial plants, were harvested for analysis and the remainder were placed in two glass inclosed chambers. The air in one chamber was maintained at 95 and in the other at 35 per cent. of saturation. The temperature was 70° F. (21° C.) in both cases. These conditions were constant day and night, with a variation in temperature of not more than $\pm 1°$ and in humidity of not more than ± 1.8 per cent. Sunlight and carbon dioxide supply were variable but the same for both humidity treatments. Condensation of moisture on the glass of the chambers did not occur as the surrounding air outside the chambers was not allowed to drop below 90° F.

Each chamber accommodated 20 pots, or 100 plants. After 12 hours under the humidity conditions indicated, half of the plants in each chamber received a complete or plus-NO_3 nutrient solution and the remainder continued to receive a solution lacking nitrogen. The nutrient solutions were applied daily, as already described, and the pans were kept constantly full of distilled water. In the low humidity chamber the surface sand at the top of the pots became dry to a depth of about 0.5 inch, but in the vicinity of the root systems the moisture was found to be in each case approximately 49 per cent. of the total water-holding capacity of the sand. With the same nutrient treatment, differences observed in growth and metabolism were brought about solely as the result of differences in humidity, although obviously all factors of environment influenced the response obtained.

Methods of chemical analysis of the tomato plants have already been described (**11**).

Results

Initial plants

The initial plants which were used for analysis and others of the same lot which were employed for experimental treatment were selected from a population of about 700, and were uniform in size, quality, and appearance. They were collected for analysis and experimental treatment begun on May 31, after the plants had been grown as already described with no external nitrogen supply. At that time they exhibited all the usual symptoms of nitrogen deficiency; and like the initial plants of the temperature experiments, may be described as follows: The stems were about 25 cm. long but stiff and woody. The base of the stem was purplish blue to light yellow. The upper two or three leaves were fairly dark green but the lower leaves were distinctly yellow with purple veins. There were practically no blossoms present. The root system was unusually large in proportion to the tops and consisted of many fine white extensive roots. The plants were high

in carbohydrates but very low in all forms of organic nitrogen, and contained neither nitrate nor ammonium (tables I–IV). Cell walls of mechanical and conductive tissue were thick, and starch was observed in large quantities in all parenchymatous tissue nearly to the tip of the stem.

Plants deficient in nitrate at 35 per cent. humidity

The plants deficient in nitrate were harvested for analysis on June 8, nine days after being continuously subjected to 35 per cent. humidity. During that period there was no noticeable change in appearance and but little change in chemical composition as compared with their initial condition on May 31 (tables I–IV).

Plants which received complete nutrient solution at 35 per cent. humidity

These plants absorbed nitrate instantly and in about four hours nitrate was present in abundance in all parts of the plant (**4**). Following absorption, assimilation of nitrate undoubtedly took place, for in nine days the stems increased nearly 100 per cent. in concentration of organic nitrogen (tables III, IV). The elaboration of nitrate necessarily involves utilization of carbohydrates, and as might be expected there occurred a decrease in sugars and starch (tables I, II).

Growth of these plants, however, was extremely slow for nitrate-supplied plants at 70° F. (**11**). The stems increased in length only 5–7 cm., and although there was some expansion of apical leaves they lacked vigor and succulence. They were comparatively thick, stiff to the touch, slightly mottled green in appearance, and even lower in chlorophyll (table V) than the leaf blades of the plants grown with no nitrate but with 95 per cent. humidity. It was further found that the stomatal apertures of the low humidity plants were small and that stomata were comparatively few in newly expanded leaves (**4**).

The plants exhibited no symptoms of wilting yet gave as a whole the general impression of dryness and stiffness. Microscopic observation (**4**) also showed that there was differentiation of heavy walled xylem and mechanical tissue, practically to the embryonic stem tip. In connection with this general lack of succulence, attention is called to the fact that although the plants were not low in total assimilated nitrogen, much of it was in the form of complex comparatively immobile proteins (tables III, IV).

Plants deficient in nitrate at 95 per cent. humidity

During the 9-day period at 95 per cent. humidity, these plants with no external nitrogen supply decreased materially in all determined forms of

TABLE I

CARBOHYDRATE FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF DRY MATTER, AND DRY MATTER EXPRESSED AS PERCENTAGE OF GREEN MATTER

	PERCENTAGE HUMIDITY				
	MAY 31	JUNE 8			
	INITIAL PLANTS	MINUS–N		PLUS–NO_3	
		35	95	35	95
	%	%	%	%	%
Dry matter	14.80	15.20	13.80	11.20	10.40
Reducing sugars	8.60	6.30	5.70	4.98	2.98
Sucrose	3.51	4.45	1.05	3.75	2.05
Total sugars	12.11	10.75	6.75	8.73	5.03
Starch and dextrin	16.61	18.34	14.57	14.90	11.57
Total carbohydrates	28.72	29.09	21.32	23.63	16.60

TABLE II

CARBOHYDRATE FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

	PERCENTAGE HUMIDITY				
	MAY 31	JUNE 8			
	INITIAL PLANTS	MINUS–N		PLUS–NO_3	
		35	95	35	95
	%	%	%	%	%
Reducing sugars	1.27	0.96	0.79	0.56	0.31
Sucrose	0.52	0.67	0.14	0.42	0.21
Total sugars	1.79	1.63	0.93	0.98	0.52
Starch and dextrin	2.46	2.79	2.01	1.67	1.20
Total carbohydrates	4.25	4.42	2.94	2.65	1.72

carbohydrates, as shown in tables I and II. The percentage and quality of nitrogenous material in whole stems (tables III, IV) shows a slight increase in concentration of soluble organic nitrogen, and especially in percentage of the simpler, more mobile forms. Accompanying these changes the stems elongated from 3 to 4 cm. and lost much of the purplish blue of anthocyanin. The apical leaves also expanded, the plants became definitely darker green, and in the upper part more succulent. There was likewise found a remarkably high concentration of chlorophyll and increase in chloroplasts (**4**) in the leaf blades as compared with comparable plants lacking nitrate at 35 per cent. humidity (table V).

As might be anticipated, the stomatal apertures were found to be much more open at high than at low humidity, although they were not closed in any case (**4**).

Plants which received complete nutrient solution at 95 per cent. humidity

Absorption and translocation of nitrate, as nearly as could be determined by microscopic observation (**4**), apparently took place at about the same rate as at 35 per cent. humidity. On a percentage basis the amount of assimilated nitrogen would not appear to be greater at high than at low humidity (tables III, IV), but the plants at high humidity increased much

TABLE III

Nitrogenous fractions of whole stems expressed as percentage of dry matter

	Percentage humidity				
	May 31	June 8			
	Initial plants	Minus–N		Plus–NO_3	
		35	95	35	95
	%	%	%	%	%
Total nitrate-free N	0.530	0.570	0.600	1.388	1.340
Protein N	0.418	0.478	0.480	0.788	0.420
Nitrate-free soluble N	0.112	0.092	0.120	0.600	0.920
Basic N	0.050	0.053	0.038	0.214	0.200
Amino N	0.043	0.029	0.060	0.258	0.494
Amide N	0.014	0.009	0.022	0.072	0.134
Other N	0.005	0.001	0.000	0.056	0.092
Nitrate N	None	None	None	0.466	0.500
Total N	0.530	0.570	0.600	1.854	1.840

more in volume than did those in the dry atmosphere, and, containing about the same percentage of total organic nitrogen on a green weight basis, must therefore have synthesized a considerably greater absolute amount. Necessarily accompanying comparatively rapid nitrate assimilation there occurred a marked decrease in carbohydrates (tables I, II).

Whereas the nitrate supplied plants at 35 per cent. humidity were woody and stiff, these were of extreme succulence. The newly expanded leaves had many stomata per unit area and the apertures were relatively large. The blades were thin, of an even dark green, and high in chlorophyll (table V). Not only were these organs thin and succulent, but there was only limited differentiation of xylem and mechanical elements in the stem tissue which developed during the 9-day period at high humidity. Further

TABLE IV

NITROGENOUS FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

	PERCENTAGE HUMIDITY				
	MAY 31	JUNE 8			
	INITIAL PLANTS	MINUS–N		PLUS–NO_3	
		35	95	35	95
	%	%	%	%	%
Total nitrate-free N	0.078	0.087	0.083	0.155	0.139
Protein N	0.062	0.073	0.066	0.088	0.044
Nitrate-free soluble N	0.016	0.014	0.017	0.067	0.095
Basic N	0.007	0.008	0.005	0.024	0.021
Amino N	0.006	0.004	0.009	0.029	0.051
Amide N	0.002	0.001	0.003	0.008	0.014
Other N	0.001	0.001	0.000	0.006	0.009
Nitrate N	None	None	None	0.052	0.052
Total N	0.078	0.087	0.083	0.207	0.191

cell division at the stem tip was rapid, as is apparent from the fact that the stems elongated from 12 to 15 cm.

Correlated with these growth responses it was found (tables III, IV) that an exceptionally high percentage of the total organic nitrogen was made up of amide and comparatively simple amino acids. This is in striking contrast to the quality of organic nitrogen found in the slow growing non-succulent plants at 35 per cent. humidity. These were notably high in complex proteins.

During the brief period of these experiments the roots were not noticeably affected by any of the experimental treatments.

TABLE V

CHLOROPHYLL IN THE LEAF BLADES OF TOMATO; JUNE 8

	PERCENTAGE HUMIDITY			
	MINUS–N		PLUS–NO_3	
	35	95	35	95
	%	%	%	%
Percentage of green matter	0.087	0.102	0.093	0.125
Relative values*	88	103	94	126

* Calculated using as 100 tomato plants which were subjected to plus-nitrate treatment at 70° F. and 85 per cent. humidity (11).

Discussion

Low humidity during a period of only eight days exerted a pronounced effect that was apparent in the external appearance of the tomato plants of these experiments, in that they lacked succulence, were low in chlorophyll (table V), and grew slowly as compared with comparable plants grown at high humidity. This response seems logical, however, as transpiration is greatly accelerated under conditions of low atmospheric moisture (**6, 10**). But such a marked external effect could scarcely take place were it not accompanied by internal changes. Chemical analyses (tables I–IV) show that internal changes did occur, and that they were of a type that usually (**8, 11, 12, 14, 16**) takes place in plants under conditions which promote carbohydrate accumulation.

The plants at 35 per cent. humidity with no external nitrogen supply, and therefore unmodified in carbohydrate metabolism by nitrate assimilation (**11**), were relatively high in dry matter and correspondingly low in moisture. They contained a high percentage of carbohydrates in the stems (tables I, II) and in all other plant parts. The nitrate supplied plants at low humidity, although lower in carbohydrates, owing to assimilation of nitrate (tables III, IV), were nevertheless much higher in concentration of sugars and starch than the plants with comparable nutrient treatment but at 95 per cent. humidity.

Further contributing to the high percentage of dry matter were the relatively thick cell walls of xylem and mechanical tissue found in the plants grown with low atmospheric moisture. This seems (**2, 9, 10**) to be a very common effect of low humidity. Yet it should also be emphasized that thick cell walls were developed under conditions of high humidity when nitrate was withheld, and there was thereby (**11**) maintained in the plant a fairly high concentration of carbohydrates (tables I, II). Cell wall thickness, with the possible exception of the calcium deficient plant (**17**), seems to be intimately associated with the carbohydrate content (**8, 13, 14, 15, 16, 23**). When carbohydrates are abundant cell walls are thick and the plant is stiff; when carbohydrates are low cell walls are thin and the plant is usually succulent. A wide variety of combinations of environmental conditions may modify the carbohydrate content of plants and therefore cell wall thickness.

Although the plants of these experiments were not grown to the fruiting stage, it may be mentioned that low humidity has been said (**2, 10**) to hasten flowering and fruiting. This also seems logical, as it has been demonstrated repeatedly (**8, 13, 14, 15**) that reproduction in plants takes place under conditions where carbohydrates are available in abundance rather than where carbohydrates are deficient. It may again be pointed out, how-

ever, that by withholding nitrate, carbohydrates were maintained in high concentration in the plants even at 95 per cent. humidity (tables I, II).

When grown with a complete nutrient solution, however, the plants subjected to high atmospheric moisture were low in carbohydrates (tables I, II), especially in the newly developed tissue. They therefore produced thin cell walls. Further, new growth was rapid and the plants were extremely succulent. Coupled with a comparatively low carbohydrate and dry matter content, high percentage of moisture, rapid growth, and succulence, there was found (tables III, IV) a very high concentration of the simpler soluble forms of organic nitrogen as amino, amide, and other nitrogen but the percentage of complex, relatively immobile proteins was low. This, however, is not a situation in any respect peculiar to high humidity. The less complex water soluble forms of organic nitrogen in the plant seem generally (**1, 12, 13, 14, 21**) to be present in abundance when carbohydrates are comparatively low and the moisture content is high.

At low humidity, however, under conditions of carbohydrate accumulation, slow growth, and lack of succulence, there was found (tables III, IV) in the nitrate supplied plants a fairly high concentration of total elaborated nitrogen. Yet much of it was in the form of complex, relatively immobile, dehydrated protein. An explanation is not attempted, but the general effect of dehydration apparently might be anticipated. It may be mentioned that EMIL FISCHER and others have employed dehydration in the synthesis of polypeptides from amino acids, and that storage proteins do not appear in seeds until desiccation begins (**3**). Condensation of simple amino acids to protein seems generally (**3, 12, 13, 14**) to occur with loss in moisture and increase in carbohydrates in the plant, and is not in any sense peculiarly a result of low humidity.

PART II. APPLE

The experimental technique employed and the results obtained were much alike for both tomato and apple. A comparatively brief presentation will suffice, therefore, to show that effects of relative humidity on apple are in harmony with the responses exhibited by tomato when subjected to similar conditions of humidity.

Experimental methods

The trees employed for this work were of the Rome variety. They had been propagated in the nursery as whole root grafts and were carefully selected 24-inch whips. On April 4, 1933, after several months in a cold storage cellar, 250 uniform trees were selected, washed free of adhering soil, the fine roots removed, and the dormant trees set in nitrogen-free quartz sand, in clean 3-gallon self-draining, glazed crocks, four trees per

crock. The tops of the trees were then pruned back to a uniform height of 25 cm. above the surface of the sand and all but the most distal bud was removed. This was done to eliminate variation in the number of shoots per tree, which would have made difficult the accurate comparisons of amount and quality of growth.

The crocks were placed in shallow enamelware pans and received daily applications of nutrient solution (**20**) in quantity sufficient to flush the sand. The pans were kept full at all times by the addition of distilled water. The water level in the pans did not extend more than 1 cm. above the bottom of the inside of the crocks. The sand adjacent to the roots was thus maintained at 52 per cent. of saturation.

On June 4, after the trees had been grown in sand for two months under usual greenhouse conditions, but with minus-N solution (**11, 20**), some of them, later referred to as *initial trees,* were harvested for analysis. At the time of this analysis and on all subsequent occasions, harvests were made shortly before sunrise. The remainder of the trees were placed in the humidity chambers. The air in one chamber was maintained at 40 and in the other at 95 per cent. relative humidity, with a temperature of 75° F. in both cases.

The plant population and other conditions were essentially the same as for tomato. After 12 hours in the chambers, nitrate was applied to half the plants of each group. The methods of chemical analysis of the apple trees have already been described (**18, 20**). The *current stem* and *leaves* were the only plant parts which were analyzed macrochemically. In other portions of the trees there was little noticeable difference between comparable nutrient series during the 18-day period of these experiments.

Results

Initial trees

Although the initial trees had no external nitrogen supply when they were shifted to the humidity chambers on June 4, they were nevertheless all actively growing with no sign of terminal bud development. The current stems were about 60 cm. in length and rather dark red, except the upper third of the stem which was comparatively soft and green in color. A few of the lower leaves were slightly yellowish green although the foliage of the remainder of the stem was dark green. White fibrous roots were very abundant and extensive, but of comparatively small diameter. This is a condition frequently exhibited by apple trees and other plants if grown with a limited nitrogen supply and opportunity for carbohydrate accumulation. Starch was observed in abundance nearly to the tips of the finest roots. Nitrate was not present as there was none in the nutrient medium.

TABLE VI

CARBOHYDRATE FRACTIONS EXPRESSED AS PERCENTAGE OF DRY MATTER, AND DRY MATTER EXPRESSED AS PERCENTAGE OF GREEN MATTER IN TWO-YEAR-OLD ROME APPLE TREES

	JUNE 4	PERCENTAGE HUMIDITY			
		JUNE 22			
	INITIAL TREES	MINUS–N		PLUS–NO_3	
		40	95	40	95
LEAVES	%	%	%	%	%
Dry matter	27.00	39.50	34.50	33.00	29.00
Reducing sugars	5.83	4.27	3.15	3.22	1.70
Sucrose	1.29	2.41	1.20	1.50	0.82
Total sugars	7.12	6.68	4.35	4.72	2.52
Starch and dextrin	4.13	7.72	6.55	4.26	3.00
Total carbohydrates	11.25	14.40	10.90	8.98	5.52
CURRENT STEM					
Dry matter	26.50	39.00	34.50	33.50	29.50
Reducing sugars	1.90	2.10	1.90	1.00	0.78
Sucrose	0.85	1.00	0.55	0.27	0.11
Total sugars	2.75	3.10	2.45	1.27	0.89
Starch and dextrin	6.20	5.90	3.97	3.52	1.59
Total carbohydrates	8.95	9.00	6.42	4.79	2.48

TABLE VII

CARBOHYDRATE FRACTIONS EXPRESSED AS PERCENTAGE OF GREEN MATTER IN TWO-YEAR-OLD ROME APPLE TREES

	JUNE 4	PERCENTAGE HUMIDITY			
		JUNE 22			
	INITIAL TREES	MINUS–N		PLUS–NO_3	
		40	95	40	95
LEAVES	%	%	%	%	%
Reducing sugars	1.57	1.69	1.09	1.06	0.49
Sucrose	0.35	0.95	0.41	0.49	0.24
Total sugars	1.92	2.64	1.50	1.55	0.73
Starch and dextrin	1.11	3.05	2.26	1.41	0.87
Total carbohydrates	3.03	5.69	3.76	2.96	1.60
CURRENT STEM					
Reducing sugars	0.50	0.82	0.66	0.34	0.23
Sucrose	0.23	0.39	0.19	0.09	0.03
Total sugars	0.73	1.21	0.85	0.43	0.26
Starch and dextrin	1.64	2.30	1.37	1.18	0.47
Total carbohydrates	2.37	3.51	2.22	1.61	0.73

Associated with these growth responses and those exhibited by the aerial organs, there was found in the leaves and current stem an abundance of sugars and starch (tables VI and VII) and a moderate concentration of organic nitrogen but no nitrate (tables VIII and IX).

TABLE VIII

NITROGENOUS FRACTIONS EXPRESSED AS PERCENTAGE OF DRY MATTER IN TWO-YEAR-OLD ROME APPLE TREES

	JUNE 4	PERCENTAGE HUMIDITY			
		JUNE 22			
	INITIAL TREES	MINUS–N		PLUS–NO_3	
		40	95	40	95
LEAVES	%	%	%	%	%
Total nitrate-free N	2.73	2.42	2.56	3.10	3.40
Protein N	2.29	2.17	2.26	2.68	2.82
Nitrate-free soluble N	0.44	0.25	0.30	0.42	0.58
Nitrate N	None	None	None	None	None
CURRENT STEM					
Total nitrate-free N	1.39	0.78	0.94	1.05	1.42
Protein N	0.57	0.48	0.50	0.58	0.65
Nitrate-free soluble N	0.82	0.30	0.44	0.47	0.77
Nitrate N	None	None	None	None	None

TABLE IX

NITROGENOUS FRACTIONS EXPRESSED AS PERCENTAGE OF GREEN MATTER IN TWO-YEAR-OLD ROME APPLE TREES

	JUNE 4	PERCENTAGE HUMIDITY			
		JUNE 22			
	INITIAL TREES	MINUS–N		PLUS–NO_3	
		40	95	40	95
LEAVES	%	%	%	%	%
Total nitrate-free N	0.73	0.96	0.88	1.02	0.99
Protein N	0.62	0.86	0.78	0.88	0.82
Nitrate-free soluble N	0.11	0.10	0.10	0.14	0.17
Nitrate N	None	None	None	None	None
CURRENT STEM					
Total nitrate-free N	0.37	0.30	0.32	0.35	0.42
Protein N	0.15	0.19	0.17	0.19	0.19
Nitrate-free soluble N	0.22	0.11	0.15	0.16	0.23
Nitrate N	None	None	None	None	None

Trees deficient in nitrate at 40 per cent. humidity

The trees, under these conditions of low humidity with no external nitrogen supply, apparently ceased terminal growth almost at once. At least the current stems made no measurable increase in length, and in 5 or 6 days it was clearly evident that all the trees of this group were forming a terminal bud. Simultaneously the foliage became yellowish green and the tip of the stem much stiffer and darker red. Internally this was associated with heavy deposition of starch and thickening of cell walls, especially near the tip of the stem. At the end of 18 days the leaves and current stem had increased greatly in percentage of dry matter and carbohydrates, as shown in tables VI and VII. On a dry weight basis there occurred a decrease in concentration of nitrogen (table VIII), although there was apparently translocation of soluble organic nitrogen from the current stem to the leaves where there was seemingly condensation to proteins, as indicated in table IX. As already pointed out in case of tomato, this type of response seems frequently to occur with loss of moisture and increase in dry matter in the plant (**3, 11, 12, 13, 14, 21**).

Trees which received complete nutrient solution at 40 per cent. humidity

The trees at low humidity which were supplied with nitrate absorbed it instantaneously, and nitrate as usual was observed only in the fibrous roots. That the trees assimilated nitrate was evident by the appearance of nitrite and a noticeable decrease in starch in the fibrous roots only 20 hours after nitrate was absorbed. Two days after the complete nutrient solution was applied the foliage became darker green, although dull green, and the current stem and petioles gradually decreased in intensity of red color. During the 18-day period the trees increased 5 to 8 cm. in length of current stem, and materially in area of the leaves (near the stem tip) which were only partially expanded when the experiments were commenced. At the end of 18 days, however, all of the trees had terminal buds and shortly before that time the stem tips were rapidly becoming woody and darker red. It may be pointed out that development of terminal buds was therefore much slower than in case of the trees lacking nitrate at low humidity, also that the nitrate supplied trees were much lower in dry matter and in carbohydrates (tables VI and VII). Maturity of vegetative or reproductive organs is frequently associated with accumulation of carbohydrates.

At the time of harvesting, dry matter in the current stem was considerably higher in the trees which received nitrate under the conditions of low humidity, than in the initial trees (table VI). This would seem to be due, at least in part, to the marked increase in thickness of cell walls which

occurred especially in the upper third of the current stem. Accompanying increase in thickness of cell walls, there was a decrease in percentage of the determined carbohydrate fractions (tables VI and VII). Polysaccharides other than starch may very well have increased, however, as is indicated by the work of MURNEEK (**10a**).

In the same trees the increase in percentage dry matter in the leaves (table VI) may have been due partly to relatively rapid loss of water through transpiration under the conditions of low atmospheric moisture. There was, however, no noticeable sign of wilting of these trees nor of any of the others of these experiments. On the other hand, the cuticle of the leaves of both series at 40 per cent. humidity was comparatively thick as were also the cell walls of veins and mesophyll.

Cell walls are made up partly of cellulose and other carbohydrate materials which are derived presumably through condensation of the simpler carbohydrates, resulting in a decrease of those in storage unless supplied through new synthesis. It should also be pointed out that although nitrate assimilation is limited mainly to the fibrous roots in apple (**18, 19**), the aerial organs must eventually supply the carbohydrates utilized in the synthesis of proteins from nitrate. Therefore both of these factors, assimilation of nitrate and thickening of cell walls, would presumably involve utilization of carbohydrate fractions such as those determined (tables VI and VII). This increase of dry matter with decrease in percentage of determined carbohydrates is in contrast to the occurrence in an herbaceous plant such as tomato, where fluctuations in percentage dry matter commonly correspond with changes in concentration of the simpler metabolic carbohydrates such as sugars and starch.

The concentration of organic nitrogen on a dry weight basis (table VIII) is higher in the current stem and leaves of the trees supplied with nitrate at 40 per cent. humidity than in the trees lacking an external nitrogen supply under the same conditions of atmospheric moisture. Expressed as percentage of green weight matter, there is very little difference between the two groups of trees (table IX). It should be recalled, however, that the trees which received nitrate increased considerably more in volume than those lacking it. In both cases, accompanying the conditions of low humidity and associated with the development of terminal buds there was a marked decrease in soluble organic nitrogen and increase in the relatively complex protein fraction (table IX). A similar response of the tomato plants is described earlier in this paper.

Both groups of trees at 40 per cent. humidity exhibited stomata which were approximately closed day and night. The leaves near the tip of the stem which made all or most of their growth during the 18-day experimental period were relatively small in area when fully developed. Yet

when completely expanded these leaves had a smaller number of stomata per unit area than comparable leaves at high humidity. The palisade and spongy mesophyll of the former were also very compact, with comparatively small cells and small intercellular spaces, and had, as already mentioned, thick cell walls and cuticle.

Trees deficient in nitrate at 95 per cent. humidity

The trees under high humidity conditions with no external nitrogen supply gradually increased in rate of growth of the current stem. The foliage became darker green and there was considerable increase in number and area of leaves. When the trees were harvested for analysis on June 22, the current stems had increased in length from 8 to 12 cm. In no case was there any sign of a terminal bud. The new stem growth was of comparatively small diameter, and in the upper third of the stem it was considerably less woody than in the case of either group of trees at 40 per cent. humidity. It will also be recalled that during the same experimental period, terminal buds appeared on all the trees of both nutrient series at low humidity.

Accompanying the conditions of high atmospheric moisture and associated with continuous linear stem growth and development of new leaves, there was found in the current stem and leaves of the trees without nitrate a very much lower percentage of dry matter and carbohydrates than in the trees lacking nitrate at low humidity (tables VI and VII). The contrast in the upper third of the current stem, however, was much more striking than is indicated by the macroanalysis of whole stems. In the stem tip there was comparatively little starch and the cell walls were relatively thin. In the lower portion of the stem there was much starch, and considerable increase in thickness of cell walls had occurred since the initial observations were made on June 4. This would apparently account, at least in part, for the increase in percentage dry matter as compared with that found in the current stem of the initial trees (table VI).

The leaves of the trees which were subjected to high humidity with no external nitrogen supply present a situation that requires explanation. The analytical sample *leaves* consisted of young and old leaves. The latter contained considerable starch. In fact, separate analysis of a group of lower leaves which turned yellow and abscissed before the end of the experimental period showed them to be high in starch but extremely low in nitrogen. It may be remarked that this was the only series which exhibited abscission or severe yellowing of leaves. It would seem that the low concentration of nitrogen in these lower leaves may have been associated with early senescence. The low concentration of nitrogen may also have limited, directly or indirectly, diastatic digestion in those older leaves which did

not abscise. There would thus be maintained in the leaf sample as a whole a comparatively high percentage of dry matter and carbohydrates (tables VI and VII).

The leaves of the upper third of the stem were dark green and extremely low in starch. The young leaves (near the tip of the stem) which expanded during the period at high humidity were of relatively large area but not so thick as comparable leaves under conditions of low humidity. The cuticle and cell walls were also thinner, and the palisade and spongy mesophyll very much less compact than in case of the leaves which developed under the conditions of low atmospheric moisture. As might be anticipated, the stomata of the trees of both nutrient series were wide open during the day at 95 per cent. humidity, although closed at night.

It may again be emphasized that the trees with no external nitrogen supply stopped linear stem growth almost immediately when shifted to low humidity, and rapidly developed a terminal bud. In striking contrast, accompanying high humidity there occurred the considerable increase in volume of tops and decrease in carbohydrates in the upper leaves and current stem that has already been described, even though there was no nitrogen in the nutrient medium. Between the roots of these two groups of trees there was little, if any, difference in appearance and chemical composition.

It has already been mentioned that there was loss of nitrogen from the older leaves of the trees grown without nitrate at 95 per cent. humidity. It is also apparent that there was a decrease in soluble organic nitrogen in the current stem as shown in tables VIII and IX. Obviously the considerable increase in volume of the trees could not have occurred without a drop in concentration of total nitrogen. This is clearly indicated in table IX. It would seem that in these apple trees, as in tomato, the condition of high humidity directly or indirectly resulted in a decrease in percentage of carbohydrates, accompanying which there occurred proteolysis and increased growth as already recorded. As discussed in greater detail in Part I, hydrolysis of proteins seems frequently to follow or accompany decrease in dry matter in the plant (**5, 11, 12, 13, 14, 21**).

Trees which received complete nutrient solution at 95 per cent. humidity

The trees which received nitrate at 95 per cent. humidity absorbed it instantly and it was observed only in the fine roots. Reduction to nitrite occurred in these organs, and other observations concerning the fibrous roots were essentially the same as recorded for the trees which were supplied with the complete nutrient solution at low humidity.

The leaves became noticeably darker green about 20 hours after ap-

plication of nitrate, and at the end of the 18-day period of these experiments the foliage of even the lower leaves was almost black-green. During this interval the current stems increased 12 to 16 cm. in length. There was no sign of a terminal bud and almost all red pigmentation disappeared from the current stem, which was very much softer near the tip than in case of any of the other series.

Associated with these responses there was found to be very little starch in the upper third of the stem and there the cell walls were comparatively thin. The remainder of the current stem exhibited considerable cell wall thickening and some starch, but was very much lower in starch and had thinner cell walls than comparable portions of the trees of the other groups. The relatively low percentage of carbohydrates in the current stem and leaves is clearly indicated in tables VI and VII. In general, the lower leaves contained more starch than the tip leaves but neither was high in starch.

In structure the newly expanded but fully developed leaves were similar to comparable leaves of the trees grown without nitrate under the same conditions of high humidity. The former, however, were considerably larger in area and relatively thinner. Cell walls and cuticle were very thin and the palisade and spongy mesophyll were loosely constructed of large cells and large intercellular spaces. Stomata were wide open during the day but apparently closed at night.

Associated with relatively rapid growth, nitrate assimilation, and decrease in carbohydrates, there was a much higher percentage of soluble organic nitrogen in the current stem and leaves than in case of any of the other groups of trees harvested on June 22 (tables VIII and IX). A similar situation has frequently been observed and is discussed more fully in connection with the tomato. They apparently responded in an analogous manner. Although the trees of this group increased rapidly in volume there was maintained in the current stem and leaves a high percentage of total organic nitrogen (tables VIII and IX). Presumably there was therefore a considerable increase in absolute amount of organic nitrogen newly synthesized from nitrate.

Summary

Tomato

1. Tomato plants were grown in sand maintained at 49 per cent. of saturation in all cases. The experiments were carried on in glass inclosed chambers at a constant temperature of 70° F. (21° C.). Sunlight and carbon dioxide supply were variable but at any given time were the same for each humidity treatment. One group of plants was grown at 35 and the other at 95 per cent. humidity.

2. The plants grown with a complete nutrient solution at 35 per cent. humidity were lighter green and contained less chlorophyll than those subjected to high atmospheric moisture. They grew slowly, had relatively thick leaves, stiff stems, and in general lacked succulence. Carbohydrates were relatively high and cell walls were thick. There was a fairly high concentration of total organic nitrogen but much of it was as complex insoluble protein.

3. Comparable plants at 95 per cent. humidity were dark green and high in chlorophyll. They grew rapidly, had relatively thin leaves, and the leaf and stem tissue which developed during the period of high atmospheric moisture was comparatively thin walled and succulent. Carbohydrates were relatively low and much of the organic nitrogen was water soluble.

4. It is pointed out and shown experimentally that these responses are typical of, but not peculiar to, the respective humidity treatments.

Apple

1. Young Rome apple trees were grown in sand culture under conditions similar to those described for tomato.

2. All the trees at 40 per cent. humidity had terminal buds at the end of 18 days, although those receiving the complete nutrient solution formed them much more slowly than the trees lacking nitrate under the same conditions. Accompanying low humidity the foliage became lighter green and the current stems darker red. Associated with these responses there tended to be carbohydrate accumulation and apparently condensation of the simpler forms of organic nitrogen to complex proteins.

3. Comparable trees at 95 per cent. humidity had darker green leaves which were relatively thin. The current stems exhibited little red color and elongated rapidly even in case of the trees lacking nitrate. During the 18-day period of these experiments the current stem tips remained comparatively soft and none of the trees formed terminal buds. Correlated with these responses carbohydrates were found in relatively low concentration, there was less indication of protein condensation, and cell walls were comparatively thin.

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THE SOIL AS DIRECT SOURCE OF CARBON DIOXIDE FOR ORDINARY PLANTS[1]

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Introduction

About the middle of the nineteenth century, when the photosynthetic process in chlorophyll-bearing cells was becoming appreciated, the question naturally arose as to whether the CO_2 necessary for that process in ordinary plants might be in part derived from the soil as well as from the free air. It was soon shown that the free air is generally the main source of the CO_2 of photosynthesis in green plants with aerial exposure, and that conclusion has remained well supported up to the present time; there is now no reason to question its correctness. But some experiments were reported which indicated that a portion of the CO_2 decomposed in green leaves had been derived not from the free air about those organs but from other parts of the plant body and even from the soil. Many recent writers appear to have ignored the results of those experiments, as well as the basic physical principles involved, and it is probably safe to suppose that the free air is now generally regarded as the *only* source of CO_2 for ordinary plants. That only a relatively small portion of the CO_2 actually absorbed can be supposed to enter the plant through the roots ought not to lead to the complete neglect of root absorption of CO_2, especially when it is recalled that the soil solution has generally a considerable concentration of carbonate ion, that large amounts of water are absorbed through root systems, and that almost all of the absorbed water is regularly given off in transpiration through green tissues. We submit a brief summary of the status of this fundamental question, together with some discussion of the logical possibilities and an account of a few experiments.

To conserve space we shall refer to the carbonate ions (HCO_3^- $CO_3^=$) in aqueous solution as *dissolved* CO_2. Since gaseous CO_2 cannot penetrate to active chloroplasts (which are surrounded by and thoroughly impregnated with water), it is safe to suppose that all CO_2 decomposed in green cells must be in watery solution before its reduction may occur. It is not necessary for our present purpose to attempt to distinguish between the hypothetical carbonic acid (H_2CO_3), the carbonates, and the carbonate ions, nor is it necessary to dwell on the consideration that a very small portion of

[1] Botanical contribution from the Johns Hopkins University, no. 124. A brief abstract of portions of the material published here was presented by LIVINGSTON and BEALL at the Des Moines meeting of the American Society of Plant Physiologists. December, 1930.

the CO_2 of the plant body is surely dissolved in solvents other than water, such as oils and waxes.

We shall consider just those autotrophic plant forms that have root systems in soil or in aqueous solution while stems and leaves are exposed to the free air. Completely submerged aquatics that absorb CO_2 surely receive it exclusively from the surrounding solution,—the water of lakes, streams, the sea,—and direct absorption from the free air is naturally out of the question for them.

BOUSSINGAULT (**7**), in 1844, showed that most if not all of the carbon of the ordinary green plant is derived from the free air, but UNGER (**40**) thought that absorption from the free air would not account for all the carbon in the plant.

CORENWINDER (**13**) inclosed a part of a cut branch in a glass container with air from which CO_2 had been removed, while the remainder of the branch was in the open air. Development was more pronounced in the container. He attributed the difference to more favorable temperature in the container, and concluded that the inclosed leaves had received their CO_2 from the uninclosed part of the branch. In support of the view that CO_2 may be derived from sources other than the free air, this writer mentioned an experiment of DE SAUSSURE (**35**), in which a leafy branch, still attached to a tree, was inclosed in a glass receiver with CO_2-free air; at the end of the experiment the oxygen content of the air in the receiver was found to have been notably increased. Obviously, however, this result might have been brought about without photosynthesis in the inclosed branch, if we suppose simply that some oxygen had found its way from other branches to the inclosed one and had there passed into the air space of the receiver.

BIRNER and LUCANUS, in 1866 (**5**), found that the first effect of an introduction of CO_2 into the nutrient solution in which oats were growing was a noticeable injury to the plants, but on continued addition of CO_2 those plants showed better development and higher dry weights than similar plants grown in the same nutrient solution but without added CO_2. They concluded that an extra supply of CO_2 in the solution had a favorable influence on the production of organic material, but they considered it an open question whether the additional CO_2 was absorbed by the roots and transported to the green parts or diffused from the solution into the air and absorbed therefrom by the leaves. Long afterwards FREE (**18**) reported that a bubbling stream of CO_2 passing through nutrient solution in which buckwheat plants were growing produced injury and death. Partial recovery ensued if, after the first day, the stream of CO_2 was replaced by a stream of air, but these plants remained smaller than the controls. Poisoning may have been due to CO_2 or possibly to some other cause.

BÖHM (**6**) grew plants of scarlet-runner bean, some in sand and some

in humus soil, each group being separately inclosed in a glass jar in which was air kept free from CO_2 by the presence of an open vessel of KOH solution. Finding no more starch in the plants grown in humus soil than in the others, he concluded that the extra supply of CO_2 presumably present in the humus soil could not be drawn upon.

In 1877 MOLL (**26, 27**), working in SACHS's laboratory, carried out some experiments similar to those of BÖHM, but more elaborate, and he also reached the conclusion that all CO_2 decomposed in leaves must come from the surrounding air, not from other parts of the plant. In an adequately illuminated, healthy green leaf, still attached to its plant and surrounded by CO_2-free air in a closed container, there was no evidence of starch formation even though other parts of the same plant were exposed to air unusually rich in CO_2; nor was starch formation apparently accelerated when the inclosed leaf was in ordinary air, which must have furnished some CO_2 directly. In these experiments of BÖHM and MOLL the rate of transpiration from inclosed leaves must have been very slow.

In 1878 VINES (**42**) reported that *Ricinus*, pumpkin, and maize plants, grown in closed chambers and without CO_2 in the air surrounding their leaves, gained in size less rapidly and showed much less starch than those grown in ordinary air. Although some starch was found in stomatal guard cells and in bundle sheaths of leaves grown without an aerial supply of CO_2, VINES decided that no CO_2 could have reached these leaves from other parts of the plant. In these experiments also, water loss by transpiration must have been very slow, although the CO_2-free air of the container was renewed each morning and two or three times throughout the day. In 1886 VINES wrote (**42**, pp. 82, 83), "There is no experimental evidence which would tend to shew that this property [of absorbing CO_2] is under any circumstances possessed by plants or parts of plants which do not contain chlorophyll." . . . "Recent investigations have, however, shewn that this theory [that a portion of the CO_2 required by a plant may be absorbed by the roots from the soil] is quite untenable."

SACHS, in whose laboratory the experiments of MOLL and VINES had been performed, may have been influential in leading these experimenters to such extreme conclusions, for in 1882 he wrote (**34**, p. 352), ". . . es eine durchaus unrichtige Ansicht ist, zu glauben, dass die in der Erde gewöhnlich reichlich enthaltene Kohlensäure von der Wurzeln aufgesogen und den Blättern zugeführt werde." He also wrote (**34**, p. 374) of MOLL's logen bis auf die neueste Zeit gehegt Ansicht, als ob die Kohlensäure von experiments, "Diese Versuche beweisen, dass die von den älteren Physioden Wurzeln aus in die Blätter geführt dort assimilirt würde, durchaus ungerechtfertigt ist." SACHS may have been intent on just putting an end to the old humus theory, that all of the carbon in ordinary green plants

was derived from the soil in the form of carbon compounds; it is difficult to imagine that he failed to realize that *some* CO_2 (be it ever so little) might be conveyed from roots to leaves under suitable sets of conditions. At any rate, the experimentation with which SACHS was acquainted furnished no basis for such sweeping statements as those just quoted, nor can such statements be justified by any subsequent experiments.

A year before the publication of these sentences by SACHS, PFEFFER (**30**, p. 200) had written with characteristic carefulness, " . . . dass eine Landpflanze fast die ganze Kohlensäure aus der die assimilirenden Organe umgebenden Luft schöpft, durch die Wurzeln und überhaupt die im Boden befindlichen Theile aber den Blättern nur wenig Kohlensäure zugeführt wird," . . . "so können doch kleine Mengen Kohlensäure den assimilirenden Organen von den Wurzeln aus zugeleitet werden." . . . "Uebrigens muss die Ausgiebigkeit der Transpiration, resp. die hierdurch veranlasste Wasser- und Gasbewegung in der Pflanze von Bedeutung sein für das Quantum Kohlensäure, welches vom Boden aus in die Pflanze gelangt." It is important that PFEFFER recognized that rapid transpiration might be expected to accelerate movement of CO_2 from roots to leaves.

Like PFEFFER, EWART (**17**, p. 569) recognized the possibility that CO_2, evolved within the plant or absorbed by its roots, might reach the green tissues by the internal route and might be reduced in photosynthesis. And in his translation of PFEFFER's Pflanzenphysiologie, he introduced no editorial modification of the German statement. The EWART translation (**31**, p. 329) states: ". . . a leaf or green stem absorbs directly from the atmosphere almost the whole of the carbon dioxide which it assimilates" . . . "Minute quantities of dissolved gas [CO_2] may . . . reach the leaves from the roots. . . . Indeed, the amount derived from the roots may, when transpiration is active, be sufficient to prevent the chloroplastids in the more deeply situated tissues at the base of a green stem from losing the power of assimilating carbon dioxide when exposed to light for prolonged periods in an atmosphere free from this gas."

MACDOUGAL (**20**) found that plants differed in their reaction to an atmosphere devoid of CO_2, but concluded that such growth as was possible under these conditions was due to food already present in the plant, since (**20**, p. 541) "the growth of a plant in an atmosphere free from CO_2 practically suspends the food-forming processes." The apparatus he used was similar to that of MOLL (**26, 27**), discussed later in this paper, and there was no considerable transpiration.

No further experimentation on this question seems to have been reported until ZIJLSTRA's paper appeared in 1909 (**45**). Repeating one of MOLL's experiments, ZIJLSTRA used a number of monocotyledonous and dicotyledonous plants, including such forms as *Dahlia, Polygonum, Aesculus,*

Acer, Acorus, Zea, Triticum, Tradescantia. He exposed the tip portion of a leaf in an atmosphere free from CO_2 and supplied an abundance of this gas to another region of the same leaf. After a period of illumination of both regions starch appeared in the region that could not have received any CO_2 from the outside, indicating some movement of CO_2 through the tissue, but the distance traversed was apparently never more than a few centimeters. It was greater when the intercellular spaces of the intervening tissue were large, and conversely. In discussing ZIJLSTRA'S results, MOLL (**28**) admitted the inaccuracy of his own earlier conclusions as to the *absolute impossibility* of starch formation in one part of a leaf at the expense of CO_2 derived from an adjacent part, but he maintained (**28**, p. 668), "ZIJLSTRA'S results are, however, in complete agreement with the main result, formerly obtained by the speaker, according to which the carbon dioxide of the soil, even if it should be absorbed by the roots, cannot appreciably contribute to the synthesis of organic matter in the leaf." In ZIJLSTRA'S experiments, as in earlier ones, there was no considerable transpiration current, which might serve to accelerate movement of CO_2 from the region with external supply to the region without any such supply; what movement did occur was probably due wholly or almost wholly to simple diffusion through the tissues,—apparently largely to gas diffusion through intercellular spaces of the leaf.

MITSCHERLICH (**25**) grew oat plants in soil watered with tap water saturated with CO_2 and found no better yield from these plants than from similar ones watered with ordinary tap water. He conceived that any increased growth that might result from adding CO_2 to the soil might be due to increased solubility of soil materials, and concluded that, since his soil already possessed an abundance of CO_2, further addition did not improve growth.

In POLLACCI'S review (**32**) of earlier papers on this question, he pointed out some probable sources of error that might have led to the opinion that CO_2 is not absorbed at all by plant roots. He added a report on some new experiments, which may be summarized as follows: Maize seeds were allowed to germinate in an atmosphere free from CO_2 and the resulting plantlets were grown with their roots in nutrient solution to which CO_2 was added, while their leaves were in CO_2-free air. The plants that developed had dry weights greater than was shown for the seeds before they germinated, and POLLACCI stated that this could not have been true except by assimilation of CO_2 derived from the nutrient solution through the roots. In another experiment maple seedlings were first kept in darkness, with roots in nutrient solution to which CO_2 was added and with leaves in CO_2-free air, until sample leaves showed no trace of starch. Then these plants were exposed to natural light conditions. They developed well for several

months, and microscopic examination showed the presence of some starch in their leaves. Also, small plants of oak, maple, and horse-chestnut, rooted in humus soil and with their aerial organs in CO_2-free air, grew for several months and produced some starch in their leaves. From these experiments POLLACCI concluded that CO_2 absorbed by the roots of green plants may be the source of some carbon for starch formation in the green tissues.

In reporting a study of respiration of detached roots, CERIGHELLI (**11**, **12**) remarked that when a root is attached to the plant gaseous exchanges with the surrounding atmosphere are masked by phenomena connected with gaseous circulation within the plant; that water absorbed by roots carries CO_2 formed there to the upper parts of the plant where it becomes reduced by photosynthesis, and gases formed by respiration and photosynthesis in the aerial parts may be transferred to the roots. In his earlier paper (**11**) he stated that he had never verified CO_2 absorption by roots and thought, —contrary to POLLACCI, as he says,—that CO_2 originating in the root may play a more important rôle than is played by CO_2 derived from soil water.

In BREAZEALE'S discussion of carbon absorption by roots (**8**, p. 305) he wrote, "There are strong indications that the plant absorbs the CO_3 ion from solution by means of its roots." Nevertheless, in some experiments planned to throw light on the question as to whether carbon thus absorbed might eventually take part in the formation of carbohydrate or other complex organic compounds, he found that wheat plants grown under bell jars gave much smaller dry yields than were given by similar plants grown in the open, and he concluded (**8**, p. 307): "It seems quite evident that the plant is unable to substitute, to any appreciable extent, the carbon of the CO_3 radical absorbed by the roots for the carbon of the carbon dioxide taken in by the leaves." The reader needs to bear in mind that, despite its too general wording, this conclusion obviously refers just to the plants of the particular experiments in question; the evidence being insufficient to warrant its application to green plants in general. Furthermore, the environment of the plants under bell jars differed from the environment of those grown in the open in several ways, aside from the aerial supply of CO_2: the two environmental complexes were surely not alike with respect to illumination and to radiation in general; they presumably differed somewhat with respect to air temperature; and, above all, they must have differed greatly with respect to conditions that influence transpiration. Everything considered, these experiments appear to have no direct bearing on the broad question before us, but BREAZEALE was surely logically right in his first supposition, that at least some CO_2 must generally be absorbed by the roots of ordinary green plants.

BANAL (**2**) considered CO_2 as a "fundamental fertilizer," noting some benefits that accrue to the soil from its presence therein. It is not clear,

however, whether he thought considerable amounts of CO_2 might reach the foliage *via* root absorption and internal transport, or regarded CO_2 from the soil as reaching the leaves mainly or wholly by passing first into the air.

According to his monograph on photosynthesis of carbon compounds in plants, apparently STILES was convinced that the free air is the only possible source of CO_2 for the kinds of plants we are considering, as is indicated or at least implied by such expressions as the following (**37**, pp. 3, 40): ". . . but it was not at first realized that it [the free air] is the only source of the carbon compounds of the plant . . .;" "When, however, it was realized that all the organic material in the plant is derived from the carbon absorbed by the leaf . . .;" "the absorption of carbon by the plant takes place exclusively by the intake of carbon dioxide by the leaves and other green organs of plants." On the other hand, SPOEHR did not fail to take what must be regarded as the logically correct view in this connection, for he wrote (**36**, p. 79), "It is conceivable, however, that conditions may exist in which the carbon dioxide absorbed by the roots may contribute to the total amount of carbon dioxide reduced by the plant."

STOKLASA (**38, 39**) took the definite position that considerable amounts of the CO_2 decomposed in the leaves of ordinary plants may reach the chlorophyll-bearing tissues *via* roots and stem, having been absorbed from the soil across the generally permeable boundary of the root system; and that the supply of this substance to the green tissues may in this way sometimes be considerably greater than might be accounted for by direct absorption across aerially exposed surfaces. He held that soil bacteria play an important rôle in accelerating CO_2 production in the soil, adding CO_2 to the atmosphere about the leaves as well as providing carbonate ions in the soil solution. He wrote (**38**, p. 5): "Diese Bikarbonate werden vom Wurzelsystem der Pflanze resorbiert und den Chlorophyllapparaten zugeführt. Den Pflanzen wird auf diese Weise eine grössere Menge Kohlenstoff in Form von Kohlendioxyd geboten. . . ." He wrote also (**39**, p. 104), "Die Pflanze assimiliert den Kohlenstoff für ihre photoenergetischen Prozesse nicht nur in Form von Kohlendioxyd mittels der Chlorophyllapparate aus der Luft, sondern auch mit dem Wurzelsystem in Form von Bikarbonaten aus dem Boden."

In 1929 MARIA BERGAMASCHI (**4**) published an account of some experiments that she had carried out in POLLACCI's laboratory, and concluded that at least some of the CO_2 assimilated by plants may be absorbed through their roots. In one series of these experiments maple, linden, camellia, and oleander plants were grown with CO_2 supplied to the root surfaces but without any CO_2 in the air surrounding the leaves, light conditions being allowed to fluctuate naturally. The leaves of these plants showed much starch in the stomatal guard cells and smaller amounts in the mesophyll.

The aerial parts were inclosed in glass chambers in which CO_2-free air was renewed several times daily, but no special arrangements were made to promote rapid transpiration; therefore the transpiration rate was probably very slow. Also, as the author pointed out, it is conceivable that the starch found in these leaves may have been wholly or partly formed from CO_2 of respiration; nor is the possibility ruled out that it may have been formed from carbohydrate partly or wholly derived directly or indirectly from food present in the plants before the beginning of the experiment.

In another series of experiments BERGAMASCHI ascertained, by sampling, the average carbon content per grain of a lot of maize seed that had been selected for uniformity from a single ear, and then plants derived from seeds of that same lot were grown under natural conditions of light and darkness, as follows: (1) Roots in nutrient solution well supplied with CO_2, leaves in CO_2-free air. (2) Roots in solution without CO_2, leaves in CO_2-free air. (3) Roots in solution well supplied with CO_2, leaves in air well supplied with CO_2. (4) Roots grown in darkness, without the possibility of photosynthesis.

At the end of an experiment period of 12–14 days, the carbon content, as a percentage of the dry weight, was ascertained for the plants of each series. The resulting percentages are as follows: (1) 43.64, (2) 39.30, (3) 75.72, (4) 34.86. The percentage carbon content for the lot of seed from which the plants had been grown was 41.54, and the differences were taken to mean that photosynthesis had been most active in the plants of group 3 (with natural light and with CO_2 supplied to both roots and leaves), but that it had occurred also in those of group 1 (with natural light and with CO_2 supplied to roots only); while absence of carbon fixation in the plants wholly deprived of CO_2 (group 2) or of light (group 4) was supposed to furnish an explanation of the fact that these groups showed lower carbon percentages than the original seeds. Unfortunately this method of computation and interpretation is misleading, for the percentage carbon content of a plant does not depend solely on the actual carbon content. Indeed it is conceivable that the percentage carbon content of a plant (reckoned on the basis of dry weight) might either increase or decrease in a growth period without regard to whether the actual carbon content increased, decreased, or was maintained. BERGAMASCHI'S published data from this experiment indicate, according to computations of our own, that the average actual carbon content per plant was in all four series less than the corresponding average per seed; that is, the experiment period was apparently not long enough, even with the plants of group 3, to permit photosynthesis, after it had begun, to make up for the initial loss of carbon that had occurred during seed germination and early growth. From the data given we have computed the following values for average actual carbon losses per plant:

(1) Plants under natural conditions of light and darkness, CO_2 supplied to roots only ..... 0.0506 mg.
(2) Plants under natural conditions of light and darkness, no CO_2 supplied ..... 0.0612 mg.
(3) Plants under natural conditions of light and darkness, CO_2 supplied to both roots and leaves ..... 0.0469 mg.
(4) Plants in darkness ..... 0.0440 mg.

It appears that the two series in which the carbon content could not have been increased from without (groups 2 and 4) are characterized by the largest and the smallest carbon loss respectively, and that the plants best supplied with CO_2 (group 3) lost somewhat more carbon than those grown in darkness (group 4). Therefore no logical conclusion concerning the problem in question seems to emerge from this experiment.

A recent paper by BARBIERI (**3**) shows how very erratic some modern writing may still be about sources of carbon for green plants. BARBIERI states flatly that plants cannot break up the carbon dioxide of the air and fix its carbon. According to his idea all of the carbon assimilated in the plant body must enter it from the soil, through the root system, either as organic matter or as mineral carbonates. We submit that *Orchid Review* was not to be congratulated on the publication of that paper.

In 1931 MILLER (**24**, p. 455) stated, "There thus appears to be good evidence that a green plant may absorb carbon dioxide from the soil and thus supplement its supply from the air." In support of this view MILLER mentions papers by GODLEWSKI, MOLL (**26, 27**), CAILLETET, MOLLIARD, MAQUENNE, POLLACCI (**32**), BREAZEALE (**8**), and BANAL (**2**), but of these only those here referred to furnish any evidence really bearing on the question before us. The remaining references are concerned with absorption of CO_2 from the free air, with emanation of CO_2 from soil to air, or with absorption of complex organic substances (rather than CO_2) from the soil.

RABER (**33**) also has recognized the possibility that the soil may supply a part of the CO_2 used by the plant, saying (**33**, p. 24) that "it is necessary to revise somewhat our ideas of the importance of carbon absorption by roots."

Some a priori considerations

If we were to suppose that all CO_2 reduced in aerially exposed green leaves must have been derived directly from the surrounding air, it would be necessary to find some sort of plausible explanation as to why some CO_2 or carbonate ion might not at times enter plant roots along with other solutes of the soil solution, being thence translocated to the leaves, and/or why CO_2 reaching the green tissues in that way might not be reduced by photosynthesis. On the basis of such a supposition it would be necessary also to find reasons why some of the CO_2 derived from respiration might not follow the same course and become reduced before escaping from the plant.

Hypotheses calculated to furnish such explanations and reasons would be very difficult to imagine and they would certainly be lacking in plausibility.

There seems to be no doubt that active cells are, in general, readily permeable to CO_2, and it has been shown repeatedly that CO_2 resulting from respiration passes out from active roots into the surrounding medium under some sets of conditions. Also, the soil solution generally contains a considerable concentration of CO_2. Therefore, as far as simple diffusion is concerned, we should expect inward diffusion of CO_2 to occur whenever CO_2 pressure is greater in the soil solution than in the root, while outward diffusion should occur whenever the pressure gradient of CO_2 is in the other direction. But water absorption by roots is probably accompanied by the entrance of the CO_2 dissolved in the absorbed water; for each liter of soil water absorbed we should expect the concomitant absorption of an amount of CO_2 equal to the amount present in that volume of soil solution before its water entered the roots. When water absorption is sufficiently rapid (as with rapid transpiration), there should be no outward diffusion of CO_2 from the roots and the rate of entrance might be much more rapid than could be accounted for by simple diffusion. Under such conditions the dissolved gas is probably carried into the roots by a mass movement of soil water. If such is not the case we must suppose that the root tissues act to prevent passage of CO_2 while allowing passage of the water in which the latter was dissolved. Such an assumption would involve a sort of filtering out of CO_2 and a resultant accumulation of CO_2 in the soil solution near the absorbing root surfaces. However, since root tissues are surely readily permeable to CO_2, there is no reason for supposing that any such "filtering out" of dissolved CO_2 occurs, although some of the mineral solutes do seem to be partially "filtered out" at times, in what is called selective absorption.

If it is agreed that roots do regularly contain CO_2 or carbonate ion (whether derived from the soil or from the respiratory process), and that cell membranes and cell walls are generally permeable to CO_2, there are just three ways by which CO_2 might move internally from roots to leaves: (1) it might simply diffuse, as solute, through the tissues, without mass flow of the solvent; (2) it might move along the paths and by the processes involved in the non-vascular transport of sugar and other organic solutes; or (3) it might be carried through xylem vessels by means of the mass flow of the transpiration stream.

(1) The process of simple solute diffusion is surely too slow to account for any considerable movement of CO_2 through such long distances as generally intervene between roots and leaves. When the distances considered are short the rate of CO_2 diffusion may be significant, however, as in the experiments of ZIJLSTRA (**45**), where starch was formed in leaf tissue a few centimeters beyond the region that was supplied with CO_2 from

without. Attention has been called to ZIJLSTRA'S note that gas movement in intercellular spaces was apparently important in those experiments, and it is probable that such movement might occur at a more rapid rate than solute movement if the intercellular spaces were adequate, for the mixing action of gas convection should be more pronounced in intercellular gas-filled spaces than is the similar action of liquid convection in cell cavities filled with aqueous solution. On the whole, diffusion alone may be dismissed as probably not furnishing a means for any considerable movement of CO_2 from roots to leaves, excepting when the distances involved are only a few centimeters.

(2) There is still disagreement concerning the non-vascular paths of transport of sugar and other foods in the ordinary plant, and the exact mechanism of that movement has not yet been shown clearly enough to satisfy all students. It appears, however, that the sieve tubes of the phloem offer the only path by which solutes may move from roots to leaves (or in the opposite direction) without continuous mass flow of the solution. Many writers consider these tubes as the most probable path of movement of foods, aside from movement in the transpiration stream, and MASON and MASKELL (**22, 23**) have shown that sugar and organic nitrogenous solutes surely moved in these tubes in their cotton plants. If we suppose (with CURTIS (**15**) and with MASON and MASKELL (**23**), for example) that solute movement through sieve tubes occurs partly by simple diffusion (across the sieve plates) but mostly by convection (in the supposedly streaming or rotating plasma of the several tube segments), or by some sort of creeping along liquid or liquid-solid interfaces (**14, 29**), we have at least a hypothetical scheme by which different solutes might move at much more rapid rates than would be possible by diffusion alone, although such rates would surely be greatly surpassed by the rate of flow of the transpiration stream in the xylem vessels when transpiration is rapid. This hypothesis seems to furnish the only plausible picture of a system by means of which different solutes may move in opposite directions along the same path, either at the same rate or at different rates. For example, sugar might be moving from leaf to root while CO_2 and NO_3 (**15**) might at the same time be moving from root to leaf in the same series of tubes. Movement of solutes by simple diffusion or by convection and diffusion combined may be independent of water movement excepting that of local convection. Indeed, it has not been shown that there is much transport of water through phloem at any time, although some students hypothesize a movement of solution through that tissue (**44**, and references there given). The convection hypothesis supposes that the aqueous solution of each sieve-tube section is generally being thoroughly stirred, as it were; but movement of water from one section to the next, through the plasmodesmas of the sieve plates, can

hardly be supposed to occur at rates significantly more rapid than those of water diffusion.

(3) The sap of the transpiration stream is probably never free from CO_2 that is being carried upward toward the transpiring tissues, which are generally the tissues capable of photosynthesis. MacDougal, Overton, and Smith (**21**) found that the gases of the interior of a tree trunk might contain CO_2 at high pressure. Under conditions that promote rapid transpiration, the transpiration stream probably delivers considerable amounts of CO_2 to the green leaf cells, and it is difficult to escape the supposition that all of the CO_2 thus delivered is reduced by photosynthesis when light intensity is adequate and other conditions are suitable. Unless that were true no absorption of CO_2 from the air about the foliage would occur. When illumination is inadequate (as on dark days and at night), or when the chlorophyll apparatus is inadequate (as in chlorotic leaves), CO_2 must of course be transpired, like water vapor, passing out of the leaves into the surrounding air.

No student of photosynthesis has even suggested that CO_2 formed by respiration in green leaves is not just as truly subject to reduction as is CO_2 that enters the green tissues from the air, and it would be quite unthinkable that CO_2 reaching the leaves from other regions, including the root system, is not equally subject to reduction. We cannot plausibly suppose that the chlorophyll apparatus somehow sorts internally supplied CO_2 from CO_2 absorbed from the free air and that only the latter is reduced during periods of photosynthesis. Indeed physiologists apparently agree that CO_2 absorption from the air is just the simple physical process of inward diffusion, in accordance with a concentration gradient established by the rapid removal of CO_2 from solution in the photosynthesizing cells and from the neighboring gas spaces, it being assumed that the CO_2-supplying power of the free air is continuously sufficient to maintain such a gradient. The gradient should be reversed when photosynthesis is too slow or when it does not occur, and then CO_2 should pass from leaf to surrounding air. Only when all the CO_2 reaching the chlorophyll apparatus from within the plant is reduced by photosynthesis as rapidly as it arrives is it possible for that apparatus to draw on the aerial supply of CO_2.

The body of the ordinary green plant acts as a continuous water system connecting the soil solution and the soil atmosphere below with the free air above, and the picture thus suggested is employed by many writers to furnish at least an important part of the explanation for the upward movement of water through the plant. The same picture furnishes ample ground for supposing that CO_2 absorbed from the soil, as well as that arising from respiration in roots and other darkened tissues, moves toward transpiring surfaces in an analogous manner. Under suitable conditions

for photosynthesis, some of the H_2O and some or all of the CO_2 reaching the leaves in the transpiration stream are surely reduced, thus preventing their escape into the free air in so far as they are thus fixed.

Turning to the root system: as long as CO_2 passes inward and upward out of the water-absorbing tissues as rapidly as it enters them from the soil and is produced in them by respiration, there can be no net outward movement of this substance into the soil, but when water absorption is sufficiently slow, when the concentration of CO_2 in the soil solution is low enough, and/or when the internal supply of CO_2 (as from respiration) is sufficiently rapid, then CO_2 should diffuse outward into the soil. It may be supposed that CO_2 passes from roots to soil generally only during periods of relatively slow water absorption or of rapid respiration, as in periods of slow transpiration and relatively high temperature. Not forgetting that CO_2 may to some extent diffuse outward laterally in its upward progress, it seems likely that the transpiration stream usually carries to the foliage both CO_2 derived from the soil and CO_2 produced by respiration in the cells along its course. But we should not expect any significant supply of CO_2 to reach the green tissues in this way excepting when the transpiration rate is relatively rapid. In all recorded experiments that have failed to show that some of the CO_2 reduced by photosynthesis might reach the chlorophyll apparatus *via* roots and stem, the experimental conditions have been such as to preclude rapid transpiration and rapid flow of vascular sap.

In some of MOLL'S experiments (**26, 27**) provision was made for some transpiration; the glass jar inclosing the aerial portion of the plant and confining the air about its leaves was provided with an open dish of concentrated H_2SO_4 or of strong potash lye, or a slow current of air was passed continually through the jar. These arrangements were approximately repeated in the course of our own study, to gain some idea as to the approximate magnitude of rates of transpiration that might have been possible in MOLL'S tests. Instead of a plant, a porous-porcelain atmometer was used as a source of water vapor, and it was found that only 3 or 4 gm. of water were evaporated in 24 hours. Under these test conditions, which were at least not very different from those of MOLL'S experiments, a plant could not transpire more than a very few grams of water in a day, and a portion of that water loss would naturally occur in the hours of darkness, when CO_2 delivered to the foliage from within the plant could not be reduced. Experiments in which conditions for rapid transpiration are not provided would therefore not be expected to show much transmission of CO_2 from soil through roots and stems to leaves *via* the transpiration stream. If considerable transport of CO_2 from soil to leaves through roots and stems can occur otherwise than in the transpiration stream, which has not yet been experimentally demonstrated, such transport might also be accelerated

to some extent by rapid transpiration, as well as by high concentration of CO_2 in the soil solution.

Other conditions being suitable for health and photosynthetic activity, if the concentration of CO_2 in the soil solution is high and if transpiration is rapid, we should expect a considerable but relatively small portion of the CO_2 reduced in the green tissues to be derived from the soil and from the plant's own respiratory processes, the larger portion being absorbed from the free air. But without considerable transpiration it may well be true that almost all CO_2 reduced in ordinary green leaves comes directly from the air about them in the manner commonly described.

To secure a quantitative estimate as to what fraction of the CO_2 reduced by photosynthesis might possibly come from the soil *via* roots and stem under conditions very favorable for CO_2 absorption from the soil, we may consider, for example, some alfalfa plants grown by BRIGGS and SHANTZ (9) at Akron, Colorado. For each gram of non-aqueous material produced, these plants, on the average for the season, lost by transpiration 1068 gm. of water. Let it be supposed (a) that all CO_2 brought to the leaves by the transpiration stream during daylight periods was reduced, (b) that three-fourths of the water lost by transpiration passed through the photosynthesizing tissues in those periods, and (c) that the non-aqueous material (final dry weight) of the plant may be fairly represented in this connection by the carbohydrate unit $C_6H_{10}O_5$. Then, for each gram of $C_6H_{10}O_5$ produced the CO_2 contained in 801 gm. (three-fourths of 1068 gm.) of soil solution should have been chemically reduced. If the soil solution is supposed to have contained 0.1 gm. of CO_2 per liter, then 0.08 gm. of CO_2 should be contained in 801 gm. of soil solution and that amount derived from the soil should have been reduced for each gram of $C_6H_{10}O_5$ produced. Since the production of 1 gm. of $C_6H_{10}O_5$ requires the reduction of 1.63 gm. of CO_2, 0.08 gm. of CO_2 represents 0.049 gm. (0.08/1.63) of $C_6H_{10}O_5$. Consequently, according to these various suppositions, the plants might have received from the soil about 4.9 per cent. of the CO_2 reduced by photosynthesis. Of course the respiratory processes should account for a small (but in our sense probably not negligible) additional amount of CO_2 coming to the leaves *via* the internal route, and we conclude that 95 per cent. or less of the CO_2 reduced might have been derived from the free air under the supposed conditions.

If a correction be introduced to account for the fact that the final dry weight represents somewhat less than 0.08 gm. of CO_2 per gram of non-aqueous material (since this material may contain a lower percentage of carbon than is indicated by the formula $C_6H_{10}O_5$), then the percentage of CO_2 derived from the free air should be somewhat smaller than 95, and that percentage should be still smaller if more than three-fourths of the

total water loss by transpiration occurred through the green tissues in periods of active photosynthesis. Under semi-arid conditions it is not unusual for the diurnal rate of transpiration from healthy plants to be more than three times as great as the corresponding nocturnal rate. Finally, if transpirational water loss were greater than 1068 gm. per gram of non-aqueous material produced, or if the soil solution contained more than 0.1 gm. of CO_2 per liter, then the supply of CO_2 derived from the soil would be correspondingly greater and the contribution of the free air would be correspondingly less.

Apparently no one has attempted to measure directly the CO_2 concentration of the solution about plant roots in any experiment or in the field, but measurements of the volumetric CO_2 content of soil atmosphere in the field have been made by a number of observers. We may suppose that the soil solution is in CO_2 equilibrium with the soil atmosphere, which may be considered (**10**) as under standard barometric pressure or nearly so. For a temperature of 25°, an aqueous solution of CO_2 in equilibrium with CO_2 gas under 1 atmosphere of pressure has a molar concentration of 0.0337 (**41**, p. 78), or about 1.48 gm. of CO_2 per liter. Then a soil solution containing 0.1 gm. of CO_2 per liter (as supposed in our hypothetical example) should be in CO_2 equilibrium with a soil atmosphere whose partial pressure of CO_2 is about 0.07 atmosphere; *i.e.*, the soil atmosphere corresponding to the soil solution considered in our illustration should have a volumetric CO_2 content of about 7 per cent. That value is probably higher than would represent the surface layers of soils in general, but there is no doubt that soils in which CO_2 is being rapidly produced from the decomposition of organic material (as by bacteria and fungi) would show soil atmospheres with volumetric CO_2 percentages greater than 7, especially if CO_2 diffusion directly into the free air were retarded, as in the case of rather deep soil layers with high water content or firm packing, or both. It seems not unlikely that the roots of swamp plants (such as rice growing in well manured paddies, for example) may be surrounded by solution with CO_2 concentration at least several times as great as 0.1 gm. per liter, which we have assumed. APPLEMAN (**1**) found the soil atmosphere of Maryland agricultural soils in summer, at a depth of about 10–12 cm., to have volumetric CO_2 contents as great as 5 per cent. in some instances.

From these and similar considerations the conclusion emerges that ordinary green plants with roots in soil or solution and leaves or other transpiring organs exposed to the air probably do not usually derive from the free air *all* of the CO_2 reduced by photosynthesis, but that a *portion* of that CO_2 is generally derived from the soil and, to some extent, from the plant's own respiration; also that the portion of CO_2 not derived from the free air may amount, in some instances, to at least 5 per cent. of the total

amount reduced. Such a percentage is surely not negligible for physiological science, whatever may be true for some aspects of the technology of crop production.

Some greenhouse experiments on growth in relation to CO_2 content of soil

METHODS.—In connection with our study concerning the possible photosynthetic reduction of some CO_2 derived from the soil, a series of simple greenhouse cultures was carried out to test in a preliminary and very tentative manner the relation of growth to the CO_2 content of the culture soil. Control plants were grown with the natural supply of CO_2, while the soils of the test cultures received extra CO_2. Plants of *Lupinus albus, Lycopersicum esculentum, Dracaena sanderiana,* and *Coleus blumei* were used, those of each experiment being nearly alike at the start. The experiment period, in August and September, was 24 days for *Lycopersicum* and about 45 days for each of the other forms. Frequent height measurements were made, and the average increment of shoot elongation per plant, for the whole period, was finally secured for each treatment and for each form.

The soil used was a sandy loam containing but little organic matter. Tinned sheet-iron cylinders, 19 cm. in diameter and 22 cm. high, were used as pots and these were each provided with a porous-porcelain auto-irrigator cone (**16, 19**) to maintain the mean soil moisture content nearly constant throughout the experiment period and nearly the same for all cylinders. Each cylinder was provided also with an additional irrigator cone, without water, this being buried in the soil by the side of the first. In each of the untreated cylinders the tubes from this second cone were open to the air but in each of the others the tubes served as inlet and outlet for a continuous stream of CO_2. The cultures stood about 35 cm. apart in two horizontal east-west rows, on two parallel board shelves about 25 cm. wide, 30 cm. apart, and 1 meter above the greenhouse floor. Parallel to these shelves and directly below the opening between them was a third shelf, like the others but only about 70 cm. above the floor. On this lower shelf stood the irrigator reservoir bottles. The three shelves were supported by the angle-iron frames of greenhouse benches, from which the slate tops had been removed. The cylinders that received additional CO_2 were placed alternately with the control cylinders in each row. In no case were the leaves at all crowded. Free and rapid air circulation around and among the leaves of all cultures was insured by having the ventilators in the greenhouse roof open throughout the experiment period. Thus, the CO_2 escaping from the exposed soil surface of a treated cylinder must have been rapidly disseminated in all directions, and the CO_2 concentration in the air about the foliage of the treated cultures could not have been con-

siderably higher than that of the air about the foliage of the untreated cultures. Aside from slight shade introduced by greenhouse sash and glass, light conditions were natural.

A special CO_2 generator was devised for these experiments. Two rubber-stoppered 5-gallon bottles, each with glass-tube air inlet reaching nearly to the bottom of the bottle and glass-tube outlet in the form of a siphon, are supported above an upright glass percolator 30 cm. high. The large opening of the latter (6 cm. in diameter) is closed by means of a 3-hole rubber stopper and the percolator chamber is nearly filled with quartz fragments about 0.5 cm. in diameter, into which is plunged an upright open cylindrical glass vial 3 cm. in diameter and 5 cm. high. A larger tube arranged to maintain a constant solution level is inserted in the path of each siphon tube, to maintain under practically constant pressure the outflowing solution. The two siphon tubes are eventually led downward through the stopper into the percolator and terminate side by side within the vial. One bottle is kept supplied with M/4 aqueous solution of oxalic acid, the other with a similar solution of Na_2CO_3, and the two solutions are allowed to flow slowly together and mix in the vial. Carbon dioxide is thus generated continuously and the resulting waste solution of sodium oxalate keeps the vial filled and overflowing, while the interstices among the quartz fragments in the percolator are kept filled to a depth of about 20 cm., with a gas space above. The waste solution finally escapes below through the percolator stem into a waste conduit, so bent as to maintain the solution in the percolator chamber always at the specified level, which insures the maintenance of a corresponding hydrostatic pressure upon the gas. The latter is conducted from the percolator to the first wash bottle by means of a delivery tube leading through the third hole in the rubber stopper. Rate of delivery of CO_2 is regulated by adjusting the rate of flow of the two solutions; at the lower end of each siphon tube is a porous plug of powdered quartz resting on glass wool, quartz being added or removed until the desired rate of flow is secured. In these experiments the two large bottles were replenished about once a week, the rate of flow of each solution being approximately 2 liters per day. Not quite all of the CO_2 generated escapes through the delivery outlet, for of course the waste solution has a high concentration of dissolved CO_2. To avoid some escape of this gas into the experiment room it is desirable that the waste solution be led away and discharged outside of the room. The waste conduit might be led through an opening in the greenhouse wall, emptying out of doors on the ground below.

From the generator, CO_2 was first led through a wash bottle of water, then through the first CO_2 cone in its culture cylinder, then through a second wash bottle and the second CO_2 cone, etc. It flowed through the

series continuously, by night as well as by day, at a rate of about 100 ml. per hour. From the last CO_2 cone the gas was discharged into the greenhouse, although it might better have been conducted to the outside. Thus the CO_2 content of the greenhouse air was always presumably slightly higher than that of the air out of doors, although the ventilators were open all the time; but, as has been said, there was free air circulation around all the plants, whether or not they were receiving extra CO_2 in the soil about their roots, and the apparent vigor of the plants of any culture showed no relation to their position in the greenhouse room.

By this arrangement all the CO_2 cones were kept filled with nearly pure CO_2, which diffused outward into the surrounding soil through the moist porous-porcelain wall, thus maintaining a relatively high CO_2 concentration in the soil solution of those containers that received the gas. No attempt was made to ascertain the CO_2 concentration in the soil solution of any of these culutres, but it is clear that the soil masses of the CO_2-treated cultures must have been much richer in CO_2 than were those of the controls.

RESULTS.—Data from these experiments are shown in table I. For each kind of plant and for each of the two treatments, either 1, 2, or 4 growth values are given in Roman type, followed by their average in boldface type. Each value in Roman type represents a single plant. Each culture had two plants, excepting in the case of *Dracaena*, in which there was but a single plant in each culture. The values in the right-hand column show for each kind of plant the percentage by which the average for cultures receiving extra CO_2 exceeded the corresponding control average.

The test with dark red *Coleus* was the only one failing to indicate more

TABLE I

SHOOT ELONGATION OF POTTED PLANTS AS RELATED TO CO_2 CONTENT OF THE SOIL MASS

KIND OF PLANT	INCREMENTS OF SHOOT ELONGATION		INCREASED GROWTH APPARENTLY DUE TO EXTRA CO_2 IN THE SOIL
	WITHOUT EXTRA CO_2 IN THE SOIL	WITH EXTRA CO_2 IN THE SOIL	
Coleus blumei (dark red)	8.2, 9.9: **9.1**	6.3, 8.2, 8.6, 12.8: **9.0**	−1
Coleus blumei (green)	3.9, 4.4: **4.2**	4.9, 5.7: **5.8**	26
Lycopersicum esculentum	5.2, 5.4, 5.5, 6.0: **5.5**	3.5, 7.6, 8.2, 8.9: **7.1**	29
Dracaena sanderiana	15.6: **15.6**	17.0: **17.0**	9
Lupinus albus	2.4, 3.2: **2.8**	8.2, 9.9: **9.1**	225

rapid shoot elongation in the treated cultures than in the untreated ones. The green *Coleus* showed a growth acceleration of 26 per cent. apparently related to the CO_2 treatment; and the smaller individual index from the CO_2-treated plants (4.9 cm.) is somewhat greater than the larger index from the controls (4.4 cm.). *Dracaena* (with only one control and one treated plant) indicated but little apparent effect of CO_2 treatment, growth acceleration amounting to only 9 per cent. The averages for *Lycopersicum* show growth acceleration in the treated cultures amounting to 29 per cent., but the first treated plant was clearly erratic (as was shown also by its general appearance) and the apparent CO_2 acceleration would be still greater if the first growth index of the treated group (3.5 cm.) were neglected in computing the average; if that were done the smallest individual value from the remaining three cultures (7.6 cm.) would be markedly greater than the largest individual value from the four controls (6.0 cm.). *Lupinus* showed by far the most pronounced difference between treated and untreated cultures; the extra supply of CO_2 in the soil of the former apparently accelerated growth by 225 per cent.; in this case again the smaller individual index value from the treated culture (8.2 cm.) is much greater than the larger value from the control culture (3.2 cm.).

Observations on the general appearance of the plants led to the same conclusion as is brought out by height measurements, namely, that (excepting the red *Coleus*) those plants whose roots were in soil with high CO_2 content were generally more vigorous than those whose soil received no extra CO_2, the difference being remarkably striking for *Lupinus;* less so for *Lycopersicum* and the green *Coleus;* and not very striking, but still noticeable, for *Dracaena.*

Such a limited series of results as this is to be considered with respect to the general direction of indications rather than with respect to the actual magnitudes of the apparent accelerations shown. Whatever may be the outcome of further experimental study, it is apparent that, at least under some sets of conditions, ordinary green plants may grow more vigorously if their soil solution is provided with an unusually high concentration of CO_2. Whether the growth accelerations shown by these few preliminary tests were either partially or wholly related to an extra supply of CO_2 absorbed by the roots from the soil, and transmitted to the leaves through the stems, cannot of course be definitely stated. In the tests with *Lupinus,* and perhaps also in those with the green *Coleus* and with *Lycopersicum,* it does not seem probable that all of the apparent difference between the CO_2-treated plants and the untreated ones was due merely to a difference in the rate of CO_2 supply to the green tissues; but it is of course possible that the CO_2 treatment may have been effective in other ways. For example, the supply of mineral nutrients in the soil (the so-called "plant

foods," which are not foods in any but the most careless sense) may have been somewhat greater in the treated cultures, perhaps due to more pronounced solvent action in the CO_2-treated soils; or the extra supply of CO_2 may perhaps have influenced the activity of soil organisms or nodule bacteria. These few experiments do not warrant any attempt to present here an analytical discussion of the various logical possibilities.

Summary and conclusion

1. On *a priori* grounds it appears that some of the CO_2 reduced in the photosynthesizing tissues of ordinary green plants must reach those tissues from the interior of the plant body even when transpiration is slow. A portion of the CO_2 thus supplied should arise from the plant's own respiration, but another portion should derive from direct absorption from the soil through roots. It is calculated that, with high concentration of CO_2 in the soil solution, with rapid transpiration, and with internal as well as external conditions otherwise favorable, a plant may receive from the soil as much as 5 per cent., or even more, of the CO_2 fixed in photosynthesis. There are no adequate grounds for supposing that the supply of CO_2 is always, or even generally, exclusively derived directly from the free air.

2. The transpiration stream may be considered as the most probable means of CO_2 transport from roots to leaves, although considerable transport may perhaps occur through phloem, as sugar is apparently transported either upward or downward. A few experiments have been reported as failing to show that considerable amounts of the CO_2 reduced in photosynthesis reached the leaves from within the plant, but transpiration was necessarily very slow in all these instances, because of the experimental arrangements. Satisfactorily controlled experiments with CO_2 supplied only to the plant roots and with rapid transpiration have yet to be reported.

3. A few pot experiments are described, which were carried out under greenhouse conditions in summer at Baltimore. An unusually high concentration of CO_2 was maintained in the soil mass of some pots, by diffusion from a porous-porcelain cone continuously supplied with CO_2 from a specially devised generator, and the plants so treated were generally more vigorous than the corresponding untreated ones. Growth acceleration concomitant with this CO_2 treatment was very pronounced for plants of *Lupinus albus,* less pronounced for a green form of *Coleus blumei* and for *Lycopersicum esculentum,* slight for *Dracaena sanderiana,* and absent for a dark red form of *Coleus blumei.* Attention is called to the probability that these growth responses may not have been due wholly to accelerated absorption of CO_2 from the soil, for the CO_2 treatment doubtless involved other differences between treated and untreated cultures. Arrangements were such as to promote very free air circulation around all the plants,

to avoid the possibility of a higher concentration of CO_2 in the air around the leaves of the treated plants than in the air around the leaves of the others.

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CAUSES OF BLIND WOOD IN ROSES

DONALD S. HUBBELL

(WITH TEN FIGURES)

Introduction

It is well known that many hybrid roses and also pure species produce great numbers of non-flowering branches which are commonly called "blind shoots." With some greenhouse varieties 50 per cent. or more of the average growth in one year may consist of blind shoots. These blind shoots represent a tremendous reduction in flowering wood, and because of this reduction the underlying causes of blindness are of great importance to the commercial rose grower.

The habit of blind wood formation is especially noticeable in the forced hybrid tea rose, which is a monthly blooming plant cultivated for heavy and regular flower production. The phenomenon of blindness is noticeable, however, to a lesser extent in hybrid perpetual roses and some pure species. The difference in flowering habit between the rose classes may account for the differences in blind wood production. These differences in blooming habit are exemplified by the hybrid tea rose which is never in a purely vegetative condition, and by the hybrid perpetuals and some pure species which for two-thirds of their growing season are in a vegetative state.

The production of blind wood in the rose appears to be a varietal characteristic, because with certain varieties of hybrid tea roses, regular blooming accompanied by a minimum production of blind wood occurs. Other hybrid tea varieties produce such a high percentage of blind wood that they are worthless for commercial forcing. If blind wood could be controlled some of these varieties might become valuable, and flower production could presumably be increased in many of the varieties now in commercial use.

The occurrence of blind wood on rose plants was observed many years ago. In 1859 an anonymous writer (1) stated: "Pruning no more seems to prevent free blooming than non-pruning seems to promote it. . . . When the variety Isabella Grey was placed against a warm, sunny wall, it produced thirty strong shoots each of which terminated with blind ends."

The florists' trade journals have for many years published discussions and comments on the causes of blind wood in forced rose plants. These discussions are not based on experimental data but are offered merely as a result of observations. These trade journal discussions have helped to establish the phenomenon of blindness in roses as a problem worthy of the attention of investigators in the fields of plant research.

The propagation of rose plants from blind wood was a subject for debate for many years until CORBETT (3) apparently solved the problem in 1897. After five years of research he came to the following conclusions: "(1) rose plants propagated from flowering wood gave an average yield of 29 blooms per plant, whereas plants propagated from blind wood gave an average yield of 11½ flowers per plant; (2) there was little difference in rooting habit between cuttings made from blind and normal wood; (3) there was no apparent cumulative effect from the continual selection of cuttings from flowering wood, nor was there any marked degradation from continuous use of blind wood; (4) there was no difference in vegetative vigor between the two sets of plants."

CORBETT made no attempt to determine the causes of blind wood. Other writers, however, have suggested theories as to the cause. TAYLOR (6) observed in 1919 that too severe pruning produced much flowerless growth in certain rose plants.

An anonymous writer (2) in 1929 made the following assertion concerning the causes of blind wood on rose plants: "With shorter days and gradually diminishing sunlight, the plants get too little light, and this alone causes roses to produce blind wood. . . . Do not cut out blind wood but if they are pinched back they will go on and produce good flowers."

HOLMES (4) states: "A whole week in November without sun but with warm, foggy weather causes blind growth on the weaker shoots."

The writer has been unable to find any data concerning the causes of blind wood on roses, nor was CORBETT or any of the leading rose investigators able to add to the bibliography.

Materials and methods

PLOT TECHNIQUE

The plant materials required for this project were 104 grafted rose plants, which were produced by grafting 52 flowering and 52 blind cions of Mme. Butterfly on English Manetti stock; 240 spring propagated own-root rose plants of the variety Mme. Butterfly, which were used for the nitrate experiments; and 100 own-root, two-year-old rose plants of the variety Mme. Butterfly, which were used for the pruning and budding experiments. The material for analytical work was obtained from the 100 plants selected for the pruning and budding experiments.

The rose plants were grown in the rose house of the Iowa State College greenhouses. The rose house runs east and west and contains three benches, each of which is 5 feet wide, 72 feet long, and 6 inches deep. On July 1, 1931, these benches were filled with a black loam which had been removed from an uncultivated field. Ten pounds of superphosphate and one pound of muriate of potash were well mixed with the soil and added to

each 100 square feet of bench space. On July 7 the center bench and 6 feet of the north bench on the west end were used for nitrate experiments. Thirty-two linear feet of the south bench were devoted to the plants which were used for pruning, budding, and sample collections. The grafting experiment utilized 34 linear feet of the north bench.

Chemical analyses

All samples for chemical analyses were taken from a bench of two-year-old, own-root hybrid tea rose plants of the variety Mme. Butterfly. At the time of sampling, the blind and flowering shoots were four to five nodes in length and about 30 days old. Analytical methods as outlined by Loomis (5) were used. Collections were made on the 20th of each month from September, 1931, to May, 1932, inclusive.

Experimentation

Growth habits of rose shoots

During the progress of the experiment the growth habits of blind and flowering wood on the variety Mme. Butterfly were studied. Figures 3 and 4 are illustrations of typical examples of flowering and blind rose shoots 30 days old. Shoots similar to these were used in all the experiments described in this paper. Figure 4 shows a normal flowering shoot terminated in a bud, whereas figure 3 shows a blind shoot terminated by a leaf composed of three leaflets. The flowering shoots had approximately seven nodes when they reached maturity; on the other hand, the blind shoots seldom had more than five nodes with relatively short internodes.

The blind growth of the hybrid tea differs from the active vegetative growth of the seasonal blooming rose plants such as the hybrid perpetuals, in that there is relatively little elongation of the growing point in the blind shoot after 30 days' growth. The vegetative growth of the seasonal blooming rose plant is continuous during the growing season. This active growth results in the formation of a long shoot with an indefinite number of nodes and with the terminal bud inclosed in a rosette of leaves.

In the hybrid tea rose plant of the variety Mme. Butterfly one to three shoots may develop from the upper axillary bud at the time when flowering should occur. These new shoots may continue blind or occasionally one may develop into a flowering shoot. If one of the three shoots produces a terminal bud, it may or may not develop into a normal flower. In the event that no flower formation occurs, blind growth continues intermittently or until a flowering shoot is produced. Figure 7 shows a shoot which has terminated in blind ends for three consecutive breaks from the axillary bud just behind the blind terminal.

As a rule flowering shoots have a higher percentage of moisture and

FIGS. 1–7. Fig. 1, blind shoot 55 days old, produced by budding a bud from a flowering shoot on a blind shoot. Fig. 2, flowering shoot 55 days old, produced by budding a bud from a flowering shoot on a blind shoot. Fig. 3, typical blind shoot 30 days old. Fig. 4, typical flowering shoot 30 days old. Fig. 5, flowering shoot 55 days old, produced by budding a bud from a flowering shoot on a flowering shoot. Fig. 6, flowering shoot 55 days old, produced by budding a bud from a blind shoot on a flowering shoot. Fig. 7, blind shoot approximately six months old.

appear to be more succulent and immature than the blind shoots. On the other hand, blind shoots are more slender and elastic than the normal shoots. It is the opinion of rose growers that at least 60 per cent. of the blind shoots will produce flowers in the spring of the year.

It is a common practice to bud the continuous blooming hybrid teas on seasonal blooming rose plants such as the hybrid perpetual. In most cases the hybrid tea bud retains its everblooming habit. This relationship between the bud and the stock indicates that a bud retains its flowering characteristics regardless of the stock, provided the two are compatible.

Table I shows some of the observable differences between blind and flowering shoots of the variety Mme. Butterfly. The figures show the average differences between the two types of shoots for the forcing season and

TABLE I

DIFFERENCES BETWEEN FLOWERING AND BLIND SHOOTS

MATERIAL	LEAF SURFACE	MOISTURE	DRY MATTER	GREEN WEIGHTS PER STEM
	sq. in.	%	%	*gm.*
Normal stems	.....	86	14	1.32
Blind stems	.....	76	24	0.82
Normal leaf	12	84	16	2.50
Blind leaf	16	79	21	3.10

are expressed as differences between single shoots approximately 30 days old. The flowering shoot at 30 days of age is very immature and will approximately treble its weight and double its leaf surface by the time it reaches maturity. The blind shoot, on the other hand, has usually reached the height of its development when it is 30 days old. The average length of a mature flowering stem is 14 inches and its diameter 6 mm., while the average length of a 30-day-old flowering stem is only 6 inches with a diam-

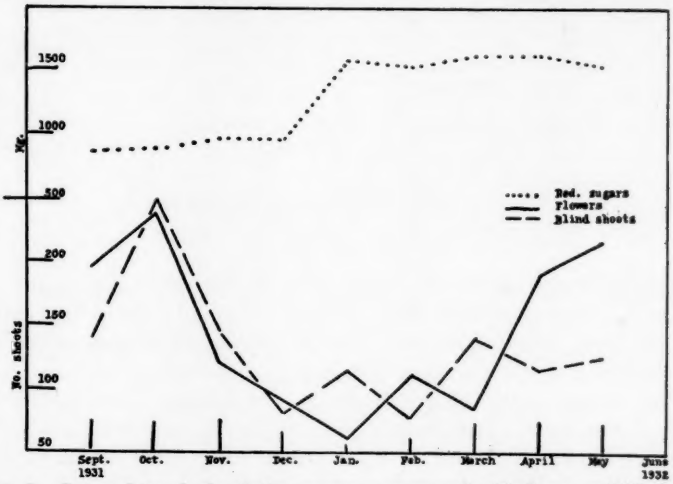

FIG. 8. Seasonal trend of reducing sugars as compared with flower and blind shoot production.

eter of 4 mm. The mature blind shoot averages 4 inches in length and has an approximate diameter of 3 mm.

The figures in table I indicate that blind and flowering shoots of an immature age differ greatly in the percentage of moisture which they contain, as well as in leaf surface. The blind shoots have approximately one and one-half times as much leaf surface as the normal shoots when they are 30 days old, and at the same time contain 10 per cent. less moisture.

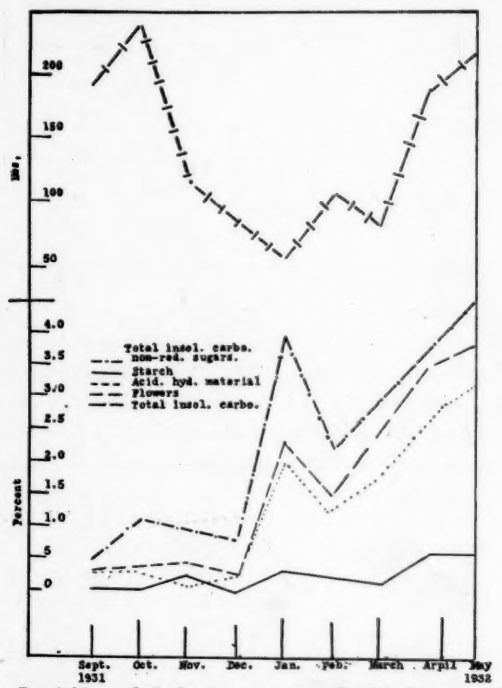

FIG. 9. Insoluble carbohydrates as compared with flower production.

The normal growth of the hybrid tea rose is of a determinate type. The nodes of 53 mature flowering stems were counted and found to average 7.3 nodes per stem. The average number of nodes on 38 immature flowering stems 30 days old was 7.1 nodes per stem. It is evident that the number of nodes present in the mature flowering stem is determined early in the growth of the shoot. Node counts made on blind shoots at 30 days of age averaged 4.5 nodes per stem and the average number of nodes for blind stems just breaking into growth was 4.9 nodes.

These data indicate that elongation of the blind shoot is stopped by the

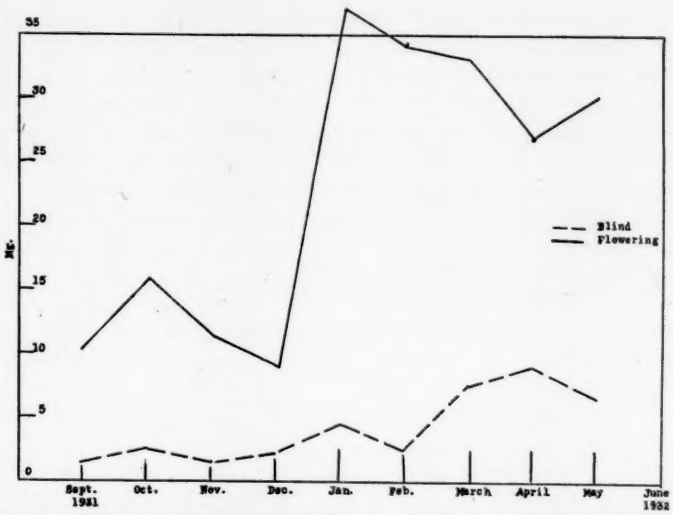

FIG. 10. C/N relationship between blind and flowering wood expressed as mg. reducing sugar per mg. non-colloidal nitrogen.

failure of the terminal flower bud to develop. The blind stem remains inactive for approximately 30 days and the first axillary bud from the tip then acts as a vegetative terminal and growth is resumed. The last two nodes on the mature flowering stem are not in prominence until they near maturity, and the attached leaves are usually in the form of misshapen bracts rather than normal leaves with from three to five leaflets. These last two nodes are not in evidence on the blind stems, since they usually have five nodes accompanied by normal leaves. It is presumed that when flower formation in the blind shoot was stopped by certain unknown factors, the formation of the last two nodes was suppressed at the same time.

BLIND WOOD AS A PHYSIOLOGICAL RATHER THAN AS A GENETIC OR PATHOLOGICAL CONDITION

GRAFTING EXPERIMENTS.—CORBETT (3) suggests that blindness in roses is an inherited characteristic. The basis for this suggestion was that plants produced from cuttings of blind wood yielded fewer flowers than plants produced from cuttings of flowering wood. Since his results were obtained from own-root plants, the writer decided to determine whether the same effects could be obtained by grafting flowering and blind cions on English Manetti.

Blind and flowering cions from Mme. Butterfly rose plants were grafted on English Manetti stock on February 1, 1931. These grafted

plants were carried in 2.5-inch rose pots until May 1, when they were shifted to 4-inch pots. On July 7, 52 uniform plants of each type of cion were selected and benched. From the time they were benched the plants were given the customary commercial rose culture. They received applications of manure water in September and October, and in February the plants were given a top dressing of fresh cow manure which was supplemented with feedings of manure water once a month until June 1. The flowers were cut and counted daily, and the blind shoots were counted at the end of each month. After the count was made the blind shoots were pinched back to the first bud back of the tip.

The yields of flowers and blind shoots of plants grafted with blind and flowering cion wood are given in table II. The main differences between

TABLE II

COMPARISON OF BLIND AND FLOWERING SHOOT PRODUCTION FROM CIONS OF BLIND AND FLOWERING WOOD

DATE	FLOWERING CIONS				BLIND CIONS			
	FLOWERS	PERCENTAGE FLOWERS	BLIND SHOOTS	PERCENTAGE BLIND	FLOWERS	PERCENTAGE FLOWERS	BLIND SHOOTS	PERCENTAGE BLIND
1931		%		%		%		%
Sept.	194	58	140	42	167	51	160	49
Oct.	239	49	251	51	237	55	194	45
Nov.	120	45	146	55	129	63	77	37
Dec.	90	52	82	48	83	61	52	38
1932								
Jan.	61	35	113	65	71	43	94	57
Feb.	108	57	81	43	95	57	72	43
March	85	38	138	62	98	59	69	41
April	188	62	115	38	155	62	97	38
May	218	63	126	37	234	69	106	31
June	283	68	132	32	278	71	112	29

the two graft types are in total shoot production and in the production of blind wood. The plants derived from flowering cion wood produced 39 more flowers and 291 more blind shoots than the plants derived from blind cions. The differences in flower production are not significant, but the differences in total shoot production are. The increase in total shoot production in the plants produced from flowering cion wood indicates that these plants were superior in vegetative vigor. The differences in vegetative vigor between the plants produced from the two types of cion wood could have been caused by the differences in cion diameter. The average diameter of the blind cion wood was 3 mm., whereas the average diameter of the flowering cion wood was 6 mm. The small diameter of the blind cion wood possibly caused more of a callous constriction than did the union

between the larger flowering cion and its stock. This constriction could have affected the plants from the blind cion in the same manner as girdling would have done; that is, a carbohydrate reserve was built up above the graft union, which stimulated flower production over vegetative growth.

It is evident from the data (table II) that there were no inherited effects from the use of blind cions in so far as the production of blind wood was concerned, nor were there any inherited effects on flower production from the use of flowering cion wood.

PRUNING AND BUDDING EXPERIMENTS.—The purpose of these experiments was to determine whether or not blindness occurred within the axillary buds of the blind shoot.

One hundred own-root, two-year-old rose plants were used for pruning experiments. The work was started in the fall of 1930 and completed June 1, 1932. Blind and flowering shoots which were approximately 30 days old were pruned to the first, second, or third axillary bud from the tip. The prunings were made whenever there were any available shoots of the proper age. For the budding experiments, buds from a 30-day-old blind shoot were budded on to mature flowering stems and buds from mature flowering stems were budded on to blind shoots. To check the results of the budding practices, normal buds were budded into normal shoots.

The results from the pruning and budding experiments are recorded in table III. The data indicate that blindness does not originate in the axillary bud but is the result of conditions arising after the bud starts to grow. The data also indicate that blindness in the bud is influenced to a marked extent by its stock.

From 200 flowering shoots, pinched to a first, second, or third axillary bud, only 3 per cent. produced blind shoots; whereas when 400 blind shoots

TABLE III

RESPONSE OF BLIND AND FLOWERING ROSE SHOOTS TO BUDDING AND PRUNING EXPERIMENTS

TREATMENT	FLOWERING SHOOTS PRODUCED	PERCENTAGE	BLIND SHOOTS PRODUCED	PERCENTAGE
		%		%
Flowering buds budded in flowering shoots	81	85.36	14	14.63
Flowering buds budded in blind shoots	6	12.50	42	87.50
Blind buds budded in flowering shoots	204	87.93	28	12.08
Flowering shoots pruned back	186	97.38	5	2.61
Blind shoots pruned back	11	2.89	380	97.10

were pinched, 97 per cent. of the new shoots produced were blind. When 100 axillary buds from flowering shoots were budded on mature stems, 15 per cent. of the new shoots formed were blind, but when 48 normal buds were budded on blind shoots, 87 per cent. of the buds were blind. Of the 232 buds taken from blind shoots and budded on to normal shoots, only 12 per cent. were blind. The 48 buds which were budded on blind shoots represent the number of buds which produced shoots. The number of buds set in blind shoots was 205. It is evident that since only 23 per cent. of the buds formed a union with the stock, there was some form of incompatibility between the two. This incompatibility was no doubt the fault of the stock, since the buds formed good unions with the flowering wood.

The preceding data indicate that blindness is a result of the stock and does not necessarily occur in the bud.[1,2] This point is illustrated by the fact that in most cases when a bud is forced into growth on a blind stem it remains blind. If, however, the bud is placed on a normal stem it produces a flower. It appears that the bud is influenced by the stock, and this fact, taken in connection with the results of the chemical analyses, indicates that blindness is a nutritional factor.

Effects of sodium nitrate applications on flower and blind wood production

Preliminary experiments indicated that the nitrogen content varied considerably between blind and flowering wood. With this variation in mind, rose plants were given varying applications of sodium nitrate to determine whether or not the quantity of soil nitrate affected the formation of blind shoots.

Two hundred and forty own-root, spring-propagated rose plants were divided among plots of high, medium, and low nitrogen content. Plots consisted of 34 square feet of bench area, separated by $1'' \times 12''$ cypress boards. Twenty carefully selected plants were planted in each plot on July 7 with alternating treatments of high, medium, and low nitrate concentrations. This plot arrangement for each treatment allowed 80 plants to be well distributed through the length of the rose house.

The high nitrogen plots received 6 pounds of sodium nitrate for every

[1] The time of flower initiation has not been determined for the rose. For this reason it was not known in the budding experiments whether the bud used was blind, flowering, or undifferentiated at the time of budding. The lack of such knowledge probably affected the budding results to some extent. The data indicate, however, that the axillary buds will produce flowering shoots provided they are grown on a flowering stem.

[2] Hubbell, D. S. A morphological study of blind and flowering rose shoots, with special reference to flower-bud differentiation. Jour. Agr. Res. 48: 91–95. 1934. This paper shows that differentiation starts to take place eight days after an axillary bud starts growth. Hence the buds in question here were in a state of undifferentiation.

TABLE IV

BLIND AND FLOWERING SHOOT PRODUCTION FROM HIGH, MEDIUM, AND LOW NITROGEN PLOTS

DATE	HIGH NITROGEN PLOT				MEDIUM NITROGEN PLOT				LOW NITROGEN PLOT			
	FLOWERS	PERCENTAGE FLOWERS	BLIND SHOOTS	PERCENTAGE BLIND	FLOWERS	PERCENTAGE FLOWERS	BLIND SHOOTS	PERCENTAGE BLIND	FLOWERS	PERCENTAGE FLOWERS	BLIND SHOOTS	PERCENTAGE BLIND
		%		%		%		%		%		%
Sept.	99	47.82	108	52.17	170	45.69	202	54.30	191	45.15	232	54.84
Oct.	154	53.65	133	46.34	219	47.50	242	53.49	238	40.43	353	59.56
Nov.	113	55.39	91	44.60	172	60.13	114	39.86	169	51.68	158	48.31
Dec.	81	57.44	60	42.55	80	51.61	75	48.38	95	45.26	115	54.76
Jan.	53	40.45	78	59.54	60	30.76	135	69.23	52	23.00	174	77.00
Feb.	96	64.00	54	36.00	111	54.67	92	45.32	95	43.37	124	56.62
March	138	64.18	77	55.81	151	50.50	148	49.49	128	41.10	181	58.89
April	144	56.25	112	43.75	180	61.39	114	38.60	119	50.00	119	50.00
May	238	72.79	89	27.21	267	65.83	139	34.17	117	52.24	107	47.76
June	301	75.82	95	24.18	332	78.07	95	21.93	167	66.82	83	33.18
	1417	61.24	897	38.76	1742	56.23	1356	43.77	1371	45.45	1646	54.55

100 square feet of bench space during the year. The nitrate was applied dry at the rate of 2 pounds per 100 square feet in the months of July, December, and February and was watered into the soil immediately after its application. The medium nitrogen plots received a total of 3 pounds of sodium nitrate, applied at the same time and in the same manner as the high nitrogen plots. The low nitrogen plots received no nitrate fertilizer.

Beginning September 1, 1931, the number of blind shoots and flowers produced on each plot was counted. The flowers were counted daily and the blind shoots were counted at the end of each month until June 30, 1932. After the count was made the blind shoots were pinched back to the first bud from the tip.

Table IV shows a correlation between the nitrate content of the soil and blind and flowering wood production. In all three fertilizer treatments the low point in total shoot production occurred in December, January, and February.

The high nitrogen plot produced the fewest flowers and the fewest blind shoots for the season. This increase in total shoot production was apparently the result of an excess of nitrate of soda. Evidence of nitrate burning was noted on the foliage throughout the season. The average percentage of blindness for the season was only 39 per cent. The medium nitrogen plot produced the most flowers and second to the lowest number of blind shoots for the season. The average percentage of blindness for the season was 44 per cent., or 5 per cent. more than the high nitrogen plot. The low nitrogen plot was lower than the medium nitrogen plot in flower production but higher in blind shoot production. The average percentage of blind shoots for the season for the low nitrogen plot was 54 per cent., or 11 per cent. higher than the medium nitrogen plot and 16 per cent. higher than the high nitrogen plot.

The data indicate that when nitrogen is applied to the soil in the form of a nitrate it has a definite effect on the production of blind wood. An increase in nitrate supply decreased blind shoot production.

The medium nitrogen plots were the only ones which produced normal and healthy plants for the entire season. The plants within the high nitrogen plots suffered from nitrate burning while they were becoming established. The low nitrogen plot produced apparently normal plants until March, when yellowing of the foliage and decreased growth gave evidence of nitrate starvation.

Blind wood as a seasonal phenomenon

Effect of illumination on blind and flowering shoot production.—Table V shows the number of blossoms and blind shoots formed for each month from September to June inclusive. The blind shoots pro-

duced for the year were almost equal in number to the flowering shoots. When there is a decrease in flower production there is a proportionate decrease in the number of blind shoots, and vice versa. The greatest decrease in total shoot production occurs during the months from November to February inclusive, and it was during these months that the hours of monthly illumination were at their minimum. It is evident from table V that decreased monthly illumination did not increase the percentages of blind wood, although decreased illumination did decrease flower production. These figures indicate that the total number of hours of sunshine may be correlated with total shoot production, in that decreased illumination results in decreased total shoot production and increased illumination brings about an increase in total shoot production. It is apparent that increased monthly illumination in the spring increased flower production over blind shoot production, and decreased monthly illumination in the fall had an equally depressing effect on both flower and blind shoot production. The correlation between sunshine and flowering is improved by lagging the light figures 30 days. This improved correlation indicates that one month's flowers are determined by the sunlight during the period of maximum development of the shoot, which occurs about 30 days before flowering. Total growth thus appears to be correlated with light conditions suitable for photosynthesis, but the April drop in percentage of blind wood remains to be explained.

SEASONAL CHANGES IN CARBOHYDRATE RESERVE OF NORMAL ROSE SHOOTS.—Inasmuch as it is generally assumed that the reserve carbohy-

TABLE V

COMPARISON BETWEEN TOTAL MONTHLY HOURS OF SUNSHINE AND BLIND AND FLOWERING SHOOT PRODUCTION

DATE	MONTHLY SUNSHINE	FLOWERING SHOOTS PRODUCED	PERCENTAGE FLOWERING SHOOTS	BLIND SHOOTS PRODUCED	PERCENTAGE BLIND SHOOTS
1931	*hrs.*		%		%
Sept.	225	194	58	140	42
Oct.	179	239	49	251	51
Nov.	108	120	45	146	55
Dec.	116	90	52	82	48
1932					
Jan.	121	61	35	113	65
Feb.	184	108	57	81	43
March	205	85	38	138	62
April	228	188	62	115	38
May	315	218	63	126	37
June	296	283	68	132	32

drate supply is associated with flower formation, it is of interest to note the reserve carbohydrate relationships which exist between the flowering rose shoots and the periods of monthly bloom.

The curve represented in figure 8 indicates the trend of flower production in relation to the reducing sugars present in the flowering shoot. The trend is toward increased reducing sugars from fall to spring; however, there are fluctuations in the reducing sugar curve which occur simultaneously with fluctuations in flower production. When there is a decrease in reducing sugars there is an increase in flower formation, and when there is a decrease in flower production there is an increase in reducing sugars.

The curve trends for the alcohol-insoluble carbohydrates are different from those of the reducing sugar curve. The curves in figure 9 show that the individual insoluble carbohydrates have the same trend, and because of this only the curve for total insoluble carbohydrate plus non-reducing sugars will be considered. In general the curves indicate that an increase in alcohol-insoluble carbohydrate is associated with an increase in flowering. A very decided variation from this trend occurred in January when flower production was at its lowest. This low point in flower production was emphasized by a decided increase in insoluble carbohydrates, an accumulation which may have been due to the use of shoots more than 30 days of age. Since growth is retarded during this season of the year, as is indicated in figure 9, the shoots may have appeared to be 30 days of age and still be a week or more older, owing to retarded growth. This sudden check in growth could be responsible for the carbohydrate accumulations in January. In February when flower production began to increase there was a decided decrease in insoluble carbohydrates. From February on the general trend of the curve indicates an increase in insoluble carbohydrate in proportion to the increase in flower production.

It is interesting to note that, previous to the decided increase of insoluble carbohydrate in January, the total shoot production was in steady decline, whereas the quantities of insoluble carbohydrate remained almost constant. It is evident from the data that the decrease in vegetative activity which occurred in January was associated with a decided increase in total carbohydrate. In this connection it is of interest to observe that the non-colloidal nitrogen figures in table VI indicate that there is an increase in non-colloidal nitrogen in December and a sudden decrease in January. The decrease in non-colloidal nitrogen together with the increase in insoluble carbohydrate gives evidence of inactivity in the normal rose shoots in January and correlates well with the decrease in flower production.

Chemical differences in flowering and blind shoots

Differences in colloidal and non-colloidal nitrogen.—The great differences in non-colloidal nitrogen noted in tables VI and VII between flowering shoots and blind shoots appear significant. The blind stems contain two or three times as much non-colloidal nitrogen as the flowering stems, whereas the differences in non-colloidal nitrogen content in the leaves are small. Since non-colloidal nitrogen is considered as the most active form, these data suggest that the low non-colloidal content in the flowering stem may have been brought about through its utilization by the actively growing tissue. The accumulation of non-colloidal nitrogen in the blind shoot indicates that there is a surplus of active nitrogenous material in a relatively inactive tissue. Since the blind tissue is making little or no growth at the end of 30 days, there is no use for the nitrogenous material and an accumulation results. The figures show a direct correlation between flower production and the non-colloidal nitrogen content of blind wood. It appears that an increase in non-colloidal nitrogen is associated with flowering. This association is probably due to the flowering of blind shoots, and it is especially noticeable during the spring months when flower production is high and blind shoot production is low.

TABLE VI

Colloidal and non-colloidal nitrogen in rose stems expressed as mg. per 100 gm. fresh weight

Material	Date of sample collection	Non-colloidal nitrogen	Colloidal nitrogen	Total nitrogen
	1931	*mg.*	*mg.*	*mg.*
Flowering stem	May	62	396	458
Blind stem		138	311	449
Flowering stem	June	97	317	414
Blind stem		180	318	498
Flowering stem	Sept.	81	299	381
Blind stem		195	289	484
Flowering stem	Oct.	55	236	294
Blind stem		181	253	434
Flowering stem	Nov.	83	364	447
Blind stem		163	240	403
Flowering stem	Dec.	104	266	370
Blind stem		159	262	422
	1932			
Flowering stem	Jan.	45	178	233
Blind stem		131	322	454
Flowering stem	Feb.	45	221	266
Blind stem		156	262	418
Flowering stem	March	51	221	273
Blind stem		121	230	351
Flowering stem	April	62	293	356
Blind stem		138	252	391
Flowering stem	May	52	446	498
Blind stem		159	265	425

The data in tables VI and VII show fluctuations in colloidal nitrogen content for both flowering and blind tissue. There are no apparent correlations between colloidal nitrogen content and blind shoot production. The fluctuations apparent in tables VI and VII remain unexplained. In November and January the increase was in favor of the flowering stems, but in January the blind stems showed an increase in colloidal nitrogen.

TABLE VII

COLLOIDAL AND NON-COLLOIDAL NITROGEN IN ROSE LEAVES EXPRESSED AS MG. PER 100 GM. FRESH WEIGHT

MATERIAL	DATE OF SAMPLE COLLECTION	NON-COLLOIDAL NITROGEN	COLLOIDAL NITROGEN	TOTAL NITROGEN
	1931	*mg.*	*mg.*	*mg.*
Flowering stem	May	52	1086	1139
Blind stem		38	1238	1276
Flowering stem	June	38	916	954
Blind stem		34	1020	1058
Flowering stem	Sept.	38	490	528
Blind stem		58	535	594
Flowering stem	Oct.	27	480	508
Blind stem		17	658	675
Flowering stem	Nov.	51	778	830
Blind stem		41	739	780
Flowering stem	Dec.	48	681	729
Blind stem		10	692	702
	1932			
Flowering stem	Jan.	62	677	739
Blind stem		45	677	722
Flowering stem	Feb.	38	633	671
Blind stem		31	740	771
Flowering stem	March	51	646	698
Blind stem		45	665	710
Flowering stem	April	34	942	977
Blind stem		34	877	912
Flowering stem	May	69	843	912
Blind stem		55	932	988

DIFFERENCES IN SOLUBLE CARBOHYDRATES.—The data for reducing sugars in stems and leaves of blind and flowering rose shoots are presented in tables VIII and IX. The percentage of reducing sugars in the flowering stems was from two to four times greater than the percentage in the blind stems. The percentages of reducing sugars for each type of stem remained constant from September to January, when a sudden increase occurred which was maintained to the month of June. It is apparent that when large quantities of reducing sugars occur in the leaves an increase is also noted in the stems. In all cases the leaves from flowering shoots were higher in reducing sugars than were the leaves from blind shoots. The data indicate that blind shoots and low reducing sugar content are associ-

ated. This relationship might be expected, because reducing sugars represent the active forms of carbohydrates and where they are present in relatively large quantities flower formation should be favored. In the case of blind shoots where there is no flower formed, reducing sugars are not present in large quantities. In the spring of the year, when flower formation is high and blind shoot production is low, there is an increase in reducing sugars in the blind shoot. When this is associated with the non-colloidal nitrogen trend, it appears that increased non-colloidal nitrogen and increased reducing sugars in blind tissue indicate an existing condition favorable to flower formation. Since this condition appears in the spring, it may be related to the observation that many blind shoots produce flowers in the spring, thus increasing total flower production and decreasing blind shoot production.

TABLE VIII

SOLUBLE AND INSOLUBLE CARBOHYDRATE IN ROSE STEMS EXPRESSED AS MG. PER 100 GM. FRESH WEIGHT

MATERIAL (STEM)	DATE OF SAMPLE COLLECTION	REDUCING SUGARS	NON-REDUCING SUGARS	TOTAL SUGARS	STARCH	ACID HYDROLYZABLE	TOTAL CARBOHYDRATE
	1931	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Flowering	May	1360	174	1534	.....	2480	4014
Blind		605	583	1188	.....	4318	5506
Flowering	June	2200	308	2508	.....	2757	5265
Blind		450	754	1204	.....	4970	6174
Flowering	Sept.	858	223	1081	.....	337	1418
Blind		281	926	1207	.....	309	1516
Flowering	Oct.	880	705	1585	.....	403	1988
Blind		420	700	1120	.....	445	1565
Flowering	Nov.	965	700	1665	218	175	2058
Blind		315	470	785	.....	375	1160
Flowering	Dec.	965	637	1638	.....	249	1887
Blind		360	1058	1358	.....	425	1783
	1932						
Flowering	Jan.	1580	1695	3275	311	2078	5664
Blind		555	950	1505	343	3052	4900
Flowering	Feb.	1535	782	2317	227	1375	3919
Blind		420	1185	1505	75	2997	4577
Flowering	March	1705	1038	2743	132	1841	4716
Blind		880	1051	1931	39	3228	5198
Flowering	April	1720	275	1995	607	2944	5546
Blind		1215	780	1940	917	4216	7073
Flowering	May	1570	691	2261	601	3287	6149
Blind		1073	1431	2504	834	4034	7372

The non-reducing sugar content for flowering and blind stems and leaves is given in tables VIII and IX and shows excessive fluctuations and variations between the two types of tissue. Leaves from blind shoots had higher percentages of non-reducing sugar than had leaves from flowering shoots.

TABLE IX

SOLUBLE AND INSOLUBLE CARBOHYDRATE IN ROSE LEAVES EXPRESSED AS MG. PER 100 GM. FRESH WEIGHT

MATERIAL (STEM)	DATE OF SAMPLE COLLECTION	REDUCING SUGARS	NON-REDUCING SUGARS	TOTAL SUGARS	STARCH	ACID-HYDROLYZABLE MATERIAL
	1931	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Flowering	May	2025	780	2805	.....	3220
Blind		1240	1350	2590	.....	4464
Flowering	June	4110	196	4306	.....	4031
Blind		1640	536	3176	.....	4736
Flowering	Sept.	435	1429	1864	.....	216
Blind		440	767	1207	.....	220
Flowering	Oct.	435	772	1207	.....	313
Blind		325	1140	1465	.....	293
Flowering	Nov.	385	1413	1798	76	120
Blind		300	992	1292	.....	113
Flowering	Dec.	480	905	1385	.....	232
Blind		465	1323	1788	.....	120
	1932					
Flowering	Jan.	795	389	1184	642	1645
Blind		785	1712	2317	700	1353
Flowering	Feb.	890	1707	2597	135	1281
Blind		570	2227	2797	.....	1317
Flowering	March	1195	1762	2957	325	976
Blind		1225	1850	3075	.....	1880
Flowering	April	880	583	1463	623	2042
Blind		815	1712	2527	640	3225
Flowering	May	965	1748	2713	666	1920
Blind		750	2109	2859	682	2626

The presence of these inactive sugars in blind tissue suggests that they are present as reserves. These reserves are associated with conditions of inactive growth and reduced flower formation. These conditions are not favorable to carbohydrate utilization and an accumulation results.

DIFFERENCES IN POLYSACCHARIDES.—The percentages indicated in tables VIII and IX show great differences in polysaccharide content between blind and flowering shoots. Both starch and acid-hydrolyzable material increased in the spring of the year in both types of tissue. The blind shoots were consistently higher in all polysaccharides. Table VII shows the seasonal trend for acid-hydrolyzable material in blind and flowering shoots. The blind stems increased from 0.3 per cent. in September to 3 per cent. in May. Again, as was the case in non-reducing sugars, there is an apparent increase in stored material associated with inactive growth and non-fruitfulness. A sudden increase in acid-hydrolyzable substances occurs in January and continues through May. There was no evident decrease through the heavy flowering season except in the fall. From September to January the leaves from flowering shoots had a higher acid-hydrolyzable content than had the

leaves from blind shoots. From January to June these conditions were reversed and the blind leaves had higher amounts of acid-hydrolyzable material than had the normal leaves. This reversal in the leaves was possibly due to the conversion of acid-hydrolyzable substances in normal leaves into translocation material. This converted material is probably represented by the increased reducing sugars present in flowering stems and leaves. Since there is no flower formation in the blind shoots, the reserve products are not utilized to the fullest extent and an accumulation is thus brought about. It is apparent from table VIII that the total carbohydrate is continually higher in the blind shoot throughout the spring. This increase takes place in January and continues through June.

CORRELATIONS BETWEEN THE SUGAR AND NITROGEN RATIOS AND FLOWERING.—A study of the curves presented in figure 10 shows that the balance between non-colloidal nitrogen and reducing sugars varies greatly for the two types of tissue. This relationship was suggested in a previous discussion under reducing sugars. It is obvious that the ratio of sugar to nitrogen is much greater in the flowering shoot than it is in the blind shoot. This suggests that the sugar value is a limiting factor in flower formation. This point is emphasized by the rise in carbohydrate relationship to nitrogen during late winter and spring. The rise is also noted in the blind shoot, although the ratio here scarcely reaches the lowest level of the ratio found in the flowering shoots. The highest level of the ratio in blind tissue occurs in the spring when blind shoot production decreases and flower formation increases. This rise in the ratio and the drop in blind wood production indicate that the increased ratio of sugar to nitrogen is correlated with flowering in blind shoots, and may be responsible for the percentage decrease in blind wood and increase in flowering. On the other hand, the increased percentage of flowering in nitrated plots and the positive correlation between the high non-colloidal nitrogen in blind wood and total flower production on the plants indicate that no simple high nitrogen, low carbohydrate relationship is responsible for the production of the vegetative blind shoots.

Discussion

Although the preceding data do not entirely solve the problem of blindness in roses, it is felt that they do yield some fundamental information which appears to have a direct bearing on the causes of blindness in the rose plant.

From the experimental data it is evident that the rose shoot has a determinate type of growth with a fairly definite number of nodes formed previous to or at the time of flower initiation. Elongation of the blind shoot ceases when the last node makes its appearance, whereas elongation con-

tinues in the flowering shoot until the flower has reached maturity. The flowering shoot has on an average two more nodes than the blind shoot, and it is assumed that the necrosis of the flower bud in blind wood also prevents the formation of the last two nodes in the blind shoot.

The physiological behavior of blind shoots gives additional information as to the causes of blind wood. The statement of CORBETT (3) that "tendencies manifested in a branch are perpetuated from generation to generation in plants propagated asexually" does not necessarily hold true with grafting processes. It was found that plants produced by grafting blind cions on English Manetti gave little or no evidence of inferiority over plants which were produced from flowering wood. These results indicate that the tendency of roses to produce blind wood is not inherited but is a growth expression dependent upon the vigor of the stock. The fact that 97 per cent. of the pruned blind shoots produced blind shoots is evidence against the practice of pruning blind shoots as a means of increasing productiveness in roses. The budding experiments indicate that the limitations of flower formation are based on the activity of stock and not on the impotency of the bud. Since 88 per cent. of the buds taken from blind shoots produced flowers when budded on to normal stocks, it seems reasonable to assume that blind shoots are capable of producing flowers in so far as the buds are concerned. A summation of the results from the pruning and budding experiments indicates that blindness is a result of the physiological condition of the stock and that selection within a clone does not affect the proportions of blind and flowering shoots.

The opinions of commercial rose growers indicate that blindness is caused by the short day length of the winter months. These opinions have not been verified by the results of this experiment. It was found that the decreasing hours of monthly illumination decreased both blind shoots and flower production; whereas an increase in illumination with spring increased flowering and slightly decreased the proportion of blind wood produced. The data indicate that reduced illumination does not stimulate blind shoot production but does have a retarding effect on the total growth of the rose plant.

Growth and differentiation in rose plants varied directly with the quantities of available nitrate in the soil. Low nitrate decreased flower production and increased blind shoot formation; whereas an increase in soil nitrate increased flower production and decreased blind shoot formation. Accompanying the effects of a high nitrate supply on blind shoot formation were the low percentages of reducing sugars found in blind shoots. This relationship between a high nitrate supply and low reducing sugar content and its apparent stimulation on differentiation does not coincide with the

conclusions of other investigators who assert that a low sugar supply accompanied by a high nitrate supply tends to stimulate vegetative growth and promote unfruitfulness.

The chemical analyses showed that flowering shoots contained larger quantities of non-colloidal nitrogen, total nitrogen, insoluble carbohydrates, and total carbohydrates. It is evident that the active forms of carbohydrates are associated with flower production, and the non-colloidal nitrogen and inactive carbohydrates are associated with blindness. There is also a low total nitrogen-total carbohydrate relationship associated with blindness. This relationship between nitrogen and carbohydrate in its relation to blind shoot production is more pronounced in the non-colloidal nitrogen and reducing sugar ratio. In the spring of the year, when flower production is increased, there is an evident increase in the carbohydrate to nitrogen ratios. It is assumed that this increase in ratio brings about a condition which is favorable to flower formation. The increased ratio possibly promotes flowering in shoots which would have otherwise become blind, and because of this the spring increase in flower production is partially accounted for.

Summary

1. A correlation between the physiological behavior and chemical differences of blind and flowering rose shoots indicates that blindness in the rose is a physiological rather than a genetical or a pathological condition.

2. Experiments combining pruning and budding indicate that blindness is a result of the stock and is not due to impotency of the buds. This point is emphasized by the differences in the chemical composition of blind and flowering wood.

3. Growth and differentiation were definitely affected by the monthly hours of illumination and the available nitrate supply, in that a decrease in illumination decreases both flower and blind shoot production while the normal increase in illumination in the spring months increases flower production more rapidly than blind shoot production.

4. With an increase in soil nitrates blind shoot formation decreases and flower production increases. With a decrease in soil nitrates blind shoot formation increases and flower production decreases.

5. The chemical analyses indicate that blindness is associated with high percentages of non-colloidal nitrogen and insoluble carbohydrates; whereas the flowering shoots contain high percentages of reducing sugars.

The writer wishes to express his appreciation to Professors B. S. PICKETT and E. C. VOLZ for their assistance with this problem. Appreciation is also extended to Dr. W. E. LOOMIS for his advice and help in the

performance of the chemical analyses, and for his valuable criticisms on the arrangement and interpretation of the data.

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RELATION BETWEEN ROOT RESPIRATION AND ABSORPTION

LETA HENDERSON

(WITH TEN FIGURES)

Introduction

Various theories have been developed to explain the nature of the absorption of water by roots, embodying what may be called imbibitional, osmotic, and passive forces (MILLER **9**). Imbibitional forces have been emphasized and shown to be of great importance by SHULL (**15**) and others. Although KUNKEL (**7**) assumed only imbibitional forces to be effective, more recent investigators agree that osmotic forces must also play an important part. But even these forces fail to explain satisfactorily the transference of water across the endodermis into the vascular cylinder. The nature of the Casparian strip seems to indicate that the transfer of water across this region is under the control of the living cells (PRIESTLEY **12, 13, 14**). Also the effect of changing environmental conditions led BLACKMAN (**1**) to believe that vital relations of the cell, involving the transfer of energy, must be concerned in absorption.

Such energy changes would of necessity be manifested at some time in the respiratory processes of the root. There is no direct evidence showing a correlation between the respirational and absorptive activity of the root except that of NEWTON (**10, 11**), who found that the evolution of carbon dioxide varied with transpiration and with the osmotic concentration of salt solution supplied in the field. The present study was made to determine more accurately the relation between these processes, using plants under strictly controlled conditions.

In order to reduce individual variations as far as possible, seed from a pure strain of corn (Funk's hybrid no. 517, Yellow Dent) was used in all the experiments. The seeds were placed in water for 12 hours, treated with 4 per cent. formalin for 10 minutes, and washed with sterile water. For germination they were placed under sterile conditions in petri dishes containing filter paper moistened with a few cubic centimeters of culture solution. This treatment resulted in normal and vigorous germination. When the radicles had reached a length of 1–2 cm. the seeds were placed on perforated sheets of cork which were floated in large dishes of Knop's solution. The solution was replaced at intervals of three to five days and continuously aerated. The corn plants were kept in the laboratory at full daylight illumination and grown in the cultures one to two weeks until needed.

Experimentation

The first experiments were carried out by means of the apparatus shown in figure 1. When the plants were 10 inches high they were placed in the experimental chamber (*A*), a 5-inch pyrex test tube with a capacity of

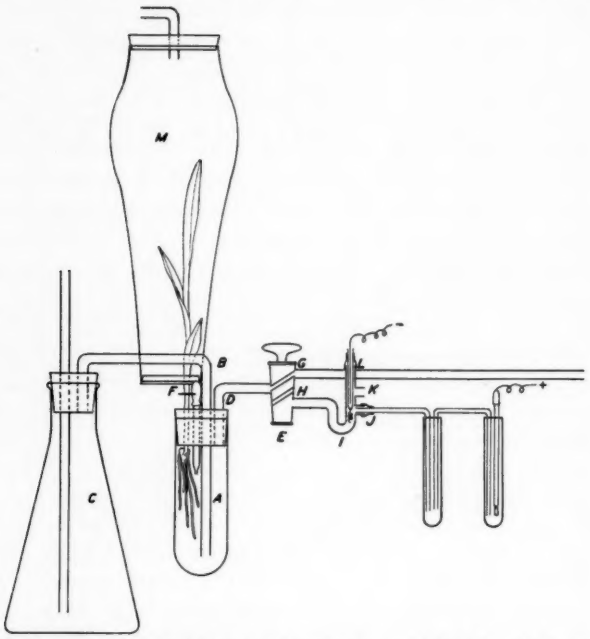

FIG. 1. Diagram of essential parts of apparatus used in determining root respiration by carbon dioxide evolution and water absorption.

90 cc. being used. A rubber stopper was fitted with an inlet tube (*B*) leading from the flask (*C*), which in turn was connected with a large bottle of culture solution. An outlet tube (*D*) led from the test tube to a three-way stopcock (*E*). A section cut from the stopper permitted the plant to be introduced without undue pressure on the stem. A small pipette (*F*) with a rubber bulb was also fitted in the stopper for use in stirring the solution before and after each reading.

The opening (*G*) of the three-way stopcock led to a graduated potometer tube of 1-mm. bore by which was measured the volume absorption during the experimental interval. The second stopcock outlet (*H*) led to the quinhydrone electrode chamber (*I*). Two glass tubes were fused into this chamber; *J* a connection for the KCl bridge, and *K* a waste outlet.

The electrode used was a platinum wire, one end of which was fused into a glass tube with mercury contact with the potentiometer circuit, and the other end coiled to carry crystals of quinhydrone when inserted into the chamber *I*. Short rubber tube connections attached the bridge and the electrode to the chamber and prevented exposure of the solutions to the air during the time of reading.

The plant was inclosed in the chamber *M*, through which air could be circulated, entering under a constant pressure at the top and escaping at the bottom. Moist or dry air could be directed through the chamber, the former passing through three towers containing water and glass wool and the latter through two towers filled with calcium chloride and equipped with dust traps of cotton wool. The apparatus was placed in a thermostat at 25° C. so that only the chamber *M* was subjected to room temperature. Although the room temperature varied from 20° to 25° C. there was seldom more than 2° of variation during a single experiment. Readings were taken at hourly intervals for from 6 to 15 hours.

The rate of transpiration and hence of absorption could be changed by passing either moist or dry air over the plant, and the accompanying effect on root respiration observed. In the first experiments the plants were placed in rain water which had been aerated for 12 hours previously. The plants were transferred from the culture dishes to the observation tubes 10 hours before readings were begun to minimize any effects due to handling. The volume absorption for any period was read directly from the calibrated potometer tube and the evolution of carbon dioxide computed for the same period from the increase in acidity of the solution, since under aerobic conditions carbonic is the only acid excreted by roots (HAAS **5**).

After mixing the culture fluid in *A* by means of the pipette *F*, a 10-cc. sample was displaced slowly through the electrode chamber. During the operation the electrode with its load of quinhydrone was elevated above the waste vent *K*. If the displacement is slow and regular, no mixing results in chamber *A*, since the solution in *C* is at the same temperature and is admitted at the end opposite the outlet. The stopcock *E* and the vent *K* are then closed and the electrode slipped down into the liquid. The quinhydrone crystals float slowly down, saturating the liquid in the electrode chamber and allowing a reading to be made. After each reading the culture around the roots was entirely replaced with fresh solution. This was insured by passing 500 cc. of fresh liquid through the culture tube until acidity determinations showed the constant and original value. All distilled water used was freshly made in a block-tin apparatus. Storage vessels used were of pyrex glass.

The carbon dioxide present may be easily determined from the acidity determination. KENDALL (**6**) has determined the dissociation constant K

of carbonic acid for concentrations up to one atmosphere to be 3.5×10^{-7}; the amount of carbon dioxide may then be determined from (H^+), K, and the volume of the solution used.

$$(1) \qquad (H^+) = \frac{a}{V}$$

where a is the degree of ionization and V is the number of liters which contain one gram mole of carbon dioxide. If K is the ionization constant, the relationship of these two may be expressed:

$$(2) \qquad K = \frac{a^2}{(1-a)V}$$

In these two equations (H^+) and K are known, so the equations may be solved simultaneously and values for a and V determined for a certain pH. From (1) we obtain:

$$(3) \qquad V = \frac{a}{(H^+)}$$

Substituting this value for V in (2) we obtain:

$$K = \frac{a^2}{(1-a)\frac{a}{(H^+)}} \text{ or}$$

$$a = \frac{K}{(H^+) + K}$$

Solving for V in (3)

$$(4) \qquad V = \frac{K}{(H^+)^2 + K(H^+)}$$

Using KENDALL'S value for K, and the (H^+) corresponding to the observed pH, the molarity of the solution may be found from V in this way:

$$C = \frac{1}{V}$$

where C is the number of gram molecules per liter. The actual weight of carbon dioxide in grams per liter may then be calculated. Since the molecular weight of carbon dioxide is 44, the total weight of carbon dioxide present is 44 C. If n represents the number of cubic centimeters of solution used, the weight of carbon dioxide in n cubic centimeters is:

$$(5) \qquad \text{Wt.} = \frac{44Cn}{1000}$$

Since the volume of 1 gram of carbon dioxide at S.T.P. is 509 cc. the volume of gas produced is:

$$(6) \qquad \text{Vol.} = \frac{509 \times 44Cn}{1000} \qquad \text{(at S.T.P.)}$$

Thus it is necessary only to substitute values in equations (4) and (6) to obtain the amount of carbon dioxide.

Results

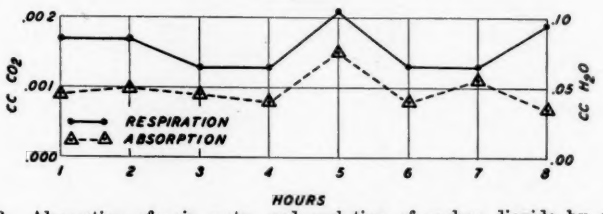

FIG. 2. Absorption of rain water and evolution of carbon dioxide by corn roots (plant 2). Leaves exposed to room conditions.

The results of the first two experiments are shown in table I. Readings were begun at 9 A. M. and the plant tops were exposed to room atmosphere, so that environmental conditions varied throughout the day. Plant 1 showed an unmistakable correlation between absorption and carbon dioxide evolution, although discrepancies appear, particularly toward the end of the experimental period. The results for plant 2 are somewhat more consistent and have been graphed in figure 2. It is evident that this plant showed a remarkably close correlation between respiration and absorption rates, and that there was no departure from this except at the final period

TABLE I

ABSORPTION OF RAIN WATER AND EVOLUTION OF CARBON DIOXIDE BY CORN ROOTS; LEAVES EXPOSED TO ROOM CONDITIONS

HOURS	PLANT 1		PLANT 2	
	ABSORPTION PER HOUR	CO_2 EVOLVED PER HOUR	ABSORPTION PER HOUR	CO_2 EVOLVED PER HOUR
	cc.	*cc.*	*cc.*	*cc.*
1	0.050	0.0017	0.040	0.0017
2	0.050	0.0012	0.050	0.0017
3	0.030	0.0012	0.045	0.0013
4	0.020	0.0007	0.040	0.0013
5	0.035	0.0022	0.075	0.0021
6	0.050	0.0022	0.040	0.0013
7	0.070	0.0007	0.055	0.0013
8	0.050	0.0004	0.035	0.0019

of observation. Variable factors evidently influenced respiration and absorption in a similar direction and to a similar degree. Thus the high rate of absorption at the fifth hour is closely paralleled by the rate of respiration, and both rates fell at the sixth hour to lower values. These high rates of absorption and respiration at the fifth hour correspond to the time of maximum illumination in the laboratory.

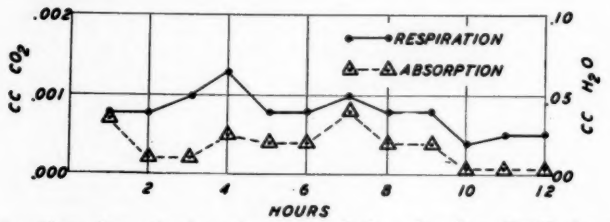

FIG. 3. Absorption of rain water and evolution of carbon dioxide by corn roots (plant 4). Leaves exposed to dry conditions for the initial eight hours.

In table II are shown the results of four experiments carried out similarly to the two previous ones, except that the tops of the plants were inclosed in dark chambers in which the humidity conditions were controlled as indicated. All plants exhibited an essentially similar relation between absorption and respiration. Plant 3 showed considerable variability but a distinct correlation between the two processes. The results for plant 4 are shown in the graph of figure 3. During the initial period of 8 hours the plant was subjected to dry air. During the 12 hours of this experiment the respiration and absorption curves show a close correlation. A high rate of absorption occurred when the plant was first exposed to dry air, but the values quickly fell to a lower level, perhaps indicating the effect of stomatal adjustment. This high initial transpiration rate is not reflected in the rate of carbon dioxide evolution. The change from dry to moist air resulted in a depression of both absorption and respiration rates, a movement which appeared after one hour and required two to reach the minimal level.

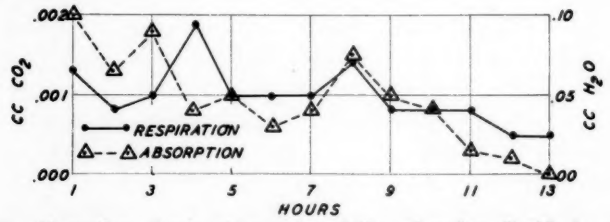

FIG. 4. Absorption of rain water and evolution of carbon dioxide by corn roots (plant 5). Leaves exposed to dry conditions for the initial eight hours.

TABLE II

ABSORPTION OF RAIN WATER AND EVOLUTION OF CARBON DIOXIDE BY CORN ROOTS; TOPS INCLOSED IN DARK CHAMBERS WITH MOIST OR DRY (*) AIR CIRCULATION

HOURS	PLANT 3		PLANT 4		PLANT 5		PLANT 6	
	ABSORPTION PER HOUR	CO_2 EVOLVED PER HOUR	ABSORPTION PER HOUR	CO_2 EVOLVED PER HOUR	ABSORPTION PER HOUR	CO_2 EVOLVED PER HOUR	ABSORPTION PER HOUR	CO_2 EVOLVED PER HOUR
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
1	0.010	0.0067	0.035*	0.0008	0.100*	0.0013	0.025	0.0011
2	0.010	0.0110	0.010*	0.0008	0.065*	0.0008	0.040	0.0007
3	0.015	0.0139	0.010*	0.0010	0.090*	0.0010	0.043	0.0008
4	0.015	0.0139	0.025*	0.0013	0.040*	0.0019	0.040*	0.0006
5	0.060*	0.0132	0.020*	0.0008	0.050*	0.0010	0.075*	0.0008
6	0.040*	0.0132	0.020*	0.0008	0.030*	0.0010	0.075*	0.0007
7	0.065*	0.0160	0.040*	0.0010	0.040*	0.0010	0.040*	0.0005
8	0.070*	0.0160	0.020*	0.0008	0.075*	0.0014	0.040*	0.0005
9	0.055*	0.0160	0.020	0.0008	0.050	0.0008		
10	0.075*	0.0132	0.005	0.0004	0.040	0.0008		
11	0.075*	0.0193	0.005	0.0005	0.015	0.0008		
12			0.005	0.0005	0.010	0.0005		
13					0.000	0.0005		

The graphs of the results obtained for plant 5 are shown in figure 4. The conditions of this experiment were identical with those of the preceding. Here again, although the curves of water absorption and of respiration are variable, they show a good correlation throughout the course of the experiment. During the initial dry period the high rate of absorption of the first hour fell through a variable course to one-third the original value, and then rose. The transfer to moist air was followed, as in the preceding experiment, by a rate of absorption which gradually fell to a minimum. The rate of evolution of carbon dioxide followed the general course of that of absorption, fluctuating during the initial period and gradually falling to a minimum during the exposure to moist air. Similarly, after the initial hour, the two processes recorded for plant 6 showed a close correlation. Since the results obtained from plants 3 and 6 are similar to those of 4 and 5, they have not been graphed.

Because the respiratory values obtained in these experiments seemed low, it was thought that buffer substances were present in the rain water, preventing a hydrogen ion concentration corresponding to the total amount of carbonic acid present. Freshly distilled and aerated water was therefore used in place of rain water. No toxic effects could be detected even over periods much longer than the duration of the experiments. The higher values obtained seemed to confirm the assumption just mentioned. Thus the values obtained in rain and distilled water cannot be compared, but only the correlation between root respiration and absorption in the two media.

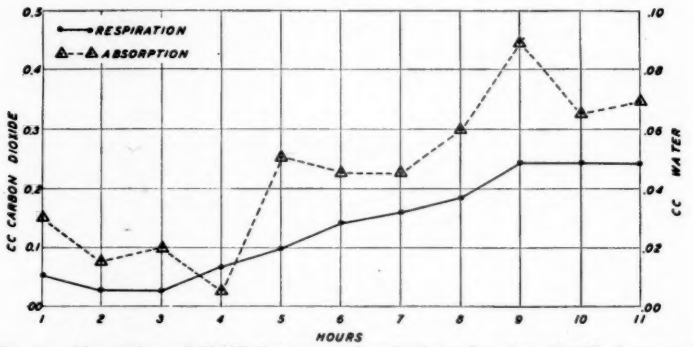

FIG. 5. Absorption of distilled water and evolution of carbon dioxide by corn roots (plant 7). Leaves exposed to moist air for the first four hours.

The results obtained when distilled water was used are shown in table III for plant 7 which was kept in a saturated atmosphere for the first four hours of the experimental period. The curves in figure 5 show a good

TABLE III

ABSORPTION OF DISTILLED WATER AND RESPIRATION OF CORN ROOTS; TOPS INCLOSED IN DARK CHAMBERS WITH MOIST OR DRY (*) AIR CIRCULATION. RESPIRATION MEASURED IN PLANT 7 BY EVOLUTION OF CARBON DIOXIDE, IN 9 BY CONSUMPTION OF OXYGEN, AND IN 8 BY BOTH PHENOMENA

Hours	Plant 7		Plant 8				Plant 9		Average for 8 and 9	
	Absorption per hour	CO_2 evolution per hour	Absorption per hour	CO_2 evolution per hour	O_2 consumption per hour	R.Q.	Absorption per hour	O_2 consumption per hour	Absorption per hour	O_2 consumption per hour
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
1	0.030	0.0532	0.045	0.1909	0.212	0.91	0.040*	0.085	0.043	0.149
2	0.015	0.0285	0.020	0.0714	0.085	0.84	0.070*	0.127	0.045	0.106
3	0.020	0.0258	0.030*	0.0777	0.127	0.61	0.080*	0.169	0.055	0.148
4	0.005	0.0692	0.035*	0.1034	0.169	0.61	0.090*	0.127	0.063	0.148
5	0.050*	0.1099	0.035*	0.1034	0.169	0.61	0.112*	0.169	0.074	0.169
6	0.045*	0.1438	0.050*	0.1034	0.169	0.61	0.070*	0.085	0.060	0.127
7	0.045*	0.1623	0.060*	0.1034	0.169	0.61				
8	0.060*	0.1873	0.060*	0.1096	0.169	0.64				
9	0.090*	0.2456	0.030*	0.1096	0.169	0.64				
10	0.065*	0.2431								
11	0.070*	0.2431								

correlation between the rates of respiration and absorption. Both rates remained at low levels during the initial four-hour moist period and rose to maximum values at the ninth hour. In this unbuffered solution the values obtained probably more nearly represent the respiratory activity of the root system than do those for the plants observed in rain water. It is significant that in both rain and distilled water there is a good correlation between the respiratory and the absorptive activities of the root system.

For following the respirational activity, the micro-Winkler method of measuring dissolved oxygen was employed either alone or with the electrometric method of carbon dioxide estimation. As used by many workers, this modification of the original Winkler method requires a sample of but 10 cc. To obtain greater accuracy the sodium thiosulphate solution was used in a concentration of N/160 instead of the usual N/40, and was added from a micro-burette. A Thompson and Miller apparatus as described by ZIMMERMAN (**16**) was found best suited to the work. The sample for analysis was withdrawn, with proper precautions against agitation and exposure to the air, through the potometer tube into the apparatus. The reagents were added immediately from the attached burette tubes and titration promptly carried out.

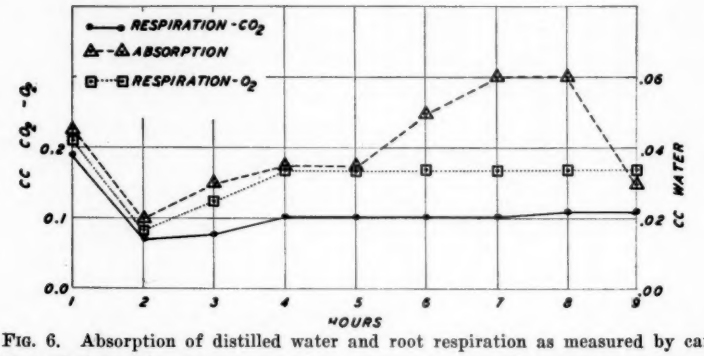

FIG. 6. Absorption of distilled water and root respiration as measured by carbon dioxide evolution and oxygen consumption (plant 8). Leaves exposed to moist conditions for the initial two hours.

The respiration of plant 8 was measured by the oxygen consumption and the carbon dioxide evolution while the root system was immersed in aerated distilled water. The results are given in table III and the curves shown in figure 6. The plant was exposed to moist air for two hours and to dry for the remainder of the experimental period. The curves show a general correlation, the discrepancy being the high rates of the sixth to eighth hours. From the initial high reading all curves fell to minimum values during the second hour, and, except for the high values just men-

tioned, rose to a moderate level for the remainder of the experiment. The curves of carbon dioxide evolution and of oxygen consumption parallel each other throughout the course of the experiment. It is evident that the rate of root respiration measured by either of the two methods gives curves which differ only in level.

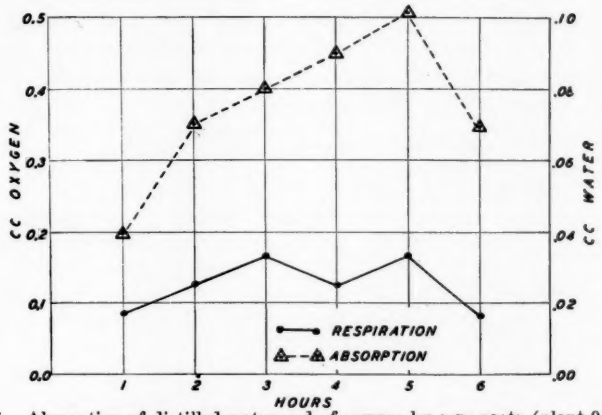

FIG. 7. Absorption of distilled water and of oxygen by corn roots (plant 9). Leaves exposed to dry conditions during the entire experiment.

The root respiration of plant 9 was followed by means of the micro-Winkler method while kept in aerated distilled water. The results are given in table III and the curves shown in figure 7. Oxygen and water absorption show a general correlation. During the initial three hours both

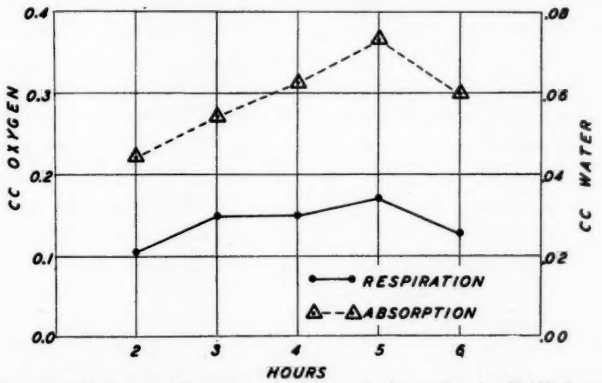

FIG. 8. General course of root respiration and absorption in distilled water (averages of plants 8 and 9).

rates increased, a movement which continued through the following two hours only for the absorption of water. Both curves rose to maxima at the fifth hour and fell to lower values at the sixth. These variations may have been due to changes in the rate of air flow over the tops, or to periodic fluctuations in the plant itself.

The average values from plants 8 and 9, showing the absorption of distilled water and of oxygen, are given in table III and the curves of the values are shown in figure 8. The first hour, during which the plants were not adjusted to the experimental condition, has not been included. The correlation between the two processes is evident.

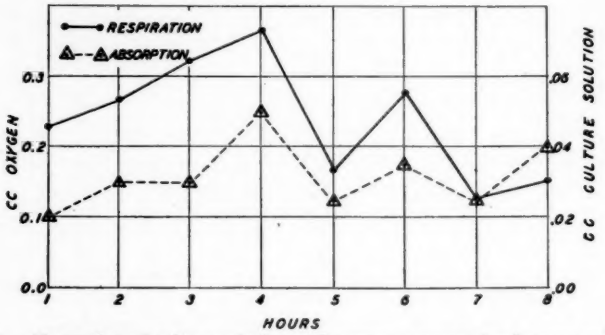

FIG. 9. Absorption of culture solution and oxygen consumption by corn roots (plant 11). Leaves surrounded by moist air for the initial hour.

Since the rain water and distilled water used in the experiments might be thought to produce abnormal root behavior, further work was carried out using culture solutions similar to those in which the plants had been grown. It is obvious that buffer action does not interfere with the determination of oxygen, and that the micro-Winkler method can be used with equal accuracy for observations in distilled water, rain water, or mineral nutrient solutions. Experiments 10, 11, and 12 were carried out in Knop's solution identical in composition with that in which the plants had been grown. The results are given in table IV. The curves of the values obtained for plant 11 are shown in figure 9 as typical of the set. It will be seen that a close correlation between respiration and absorption was shown, the two processes paralleling each other throughout the course of the experiment. From the initial low values obtained under conditions of moist air, the maximum rates of both respiration and absorption were reached at the fourth hour, after which the two processes varied simultaneously and similarly. The results obtained for plants 10 and 12 are similar to those for plant 11. The effects of moist and dry air are not so obvious as in earlier experiments.

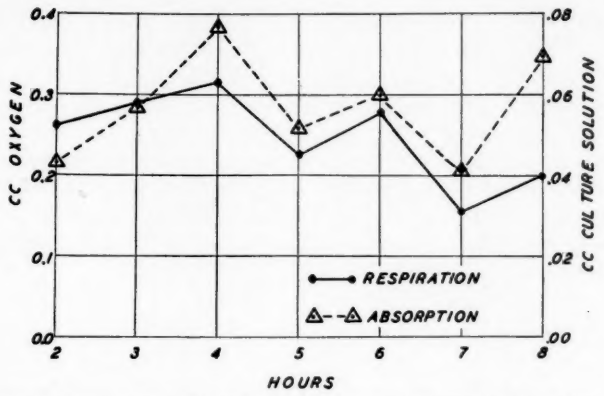

FIG. 10. General course of root respiration and absorption in culture solution. Averages of plants 10, 11, 12.

The averages for the absorption of water from the culture solution and for the consumption of oxygen are given in table IV and the curves of these values are shown in figure 10. At the first reading in experiment 12 the plant apparently had not become adjusted to the experimental conditions and hence this value is not shown in figure 10. It will be seen that the correlation between the absorption of liquid and of oxygen is close, the processes varying in parallel throughout the period.

Discussion

The thermodynamic conception of an organism is less prominent in the work of the plant physiologist than in that of workers in the field of animal physiology. Respiration, as an indication of physiological activity, may be taken as a measure of work done, even when the exact nature of this work is not evident. The thermodynamic relations of plant activities have received too little attention. Comparatively few detailed studies of the energy relations of any plant activity have been reported. Thus while we know how to evaluate respiration in terms of energy, we do not possess such knowledge concerning the use of this energy in the plant. For instance, in the fundamentally important water relations of plants many workers have hesitated to assign any endothermic physiological relation. Since the work of DUTROCHET, the osmotic mechanism is a widely accepted explanation of water absorption and transport, in spite of the fact that many of the concentration gradients along the paths do not agree in direction with that of the movement of water.

Following the work of SACHS, imbibitional forces have been accepted by

TABLE IV

ABSORPTION OF CULTURE SOLUTION AND OF OXYGEN BY CORN ROOTS; TOPS INCLOSED IN DARK CHAMBERS WITH MOIST OR DRY (*) AIR CIRCULATION

HOURS	PLANT 10		PLANT 11		PLANT 12		AVERAGE	
	ABSORPTION PER HOUR	O_2 CONSUMPTION PER HOUR	ABSORPTION PER HOUR	O_2 CONSUMPTION PER HOUR	ABSORPTION PER HOUR	O_2 CONSUMPTION PER HOUR	ABSORPTION PER HOUR	O_2 CONSUMPTION PER HOUR
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
1	0.020	0.215	0.020	0.229	0.050	0.449	0.030	0.298
2	0.050*	0.355	0.030*	0.269	0.050	0.169	0.043	0.264
3	0.090*	0.373	0.030*	0.322	0.050	0.178	0.057	0.291
4	0.110*	0.338	0.050*	0.364	0.070*	0.237	0.077	0.313
5	0.060*	0.279	0.025*	0.169	0.070*	0.237	0.052	0.228
6	0.075*	0.322	0.035*	0.279	0.070*	0.237	0.060	0.279
7	0.060*	0.245	0.025*	0.127	0.040*	0.102	0.042	0.158
8	0.120*	0.322	0.040*	0.152	0.050*	0.127	0.070	0.200

many workers as an explanation of water absorption. KUNKEL (**7**) believed these forces to be the main ones concerned in the intake of water by roots, and that osmotic forces play no part. SHULL (**15**) assigned an important rôle to imbibitional forces, but did not exclude the osmotic. According to either of these conceptions, the energy for the absorption of water would be the absorbed radiant energy necessary for vaporization and for photosynthesis in the aerial parts of the plant. Both the imbibitional and the osmotic explanations of water absorption and transport fail to explain satisfactorily exudation pressure and also the fact that an oxygen supply is necessary for normal root activity. Similarly guttation is explained with difficulty on this basis. In order to explain the transport of water under these conditions various hypotheses have been suggested. PRIESTLEY and NORTH (**14**) believed the endodermis to be the functional absorbing surface of the root; they assign to it the rôle of a semipermeable membrane, separating the intercellular cortical fluids from those of the xylem vessels. PFEFFER formulated a hypothesis based on differences in the osmotic pressure of the plasma membrane in different parts of the cell to explain the movement of water from a solution of high concentration to a low one. Although he later abandoned this as being unlikely, LEPESCHKIN (**8**) used it to explain the exudation of water by sporangiophores and by hydathodes.

More recently workers coming from the field of animal to general physiology have considered that osmotic work must be done. The actual mechanism involved is not yet clear. A secretory action may take place in the endodermis or in other tissues; but since the mechanism of glandular activity is still imperfectly understood, such a hypothesis is not very clarifying. Similarly electro-endosmosis is a possible explanation, but until more facts substantiating this have been found such an assumption is of little value.

It is apparent that irrespective of any mechanism involved, osmotic work must be done. Any system of cells along the line of water absorption and transport must maintain a gradient of increasing osmotic pressure from root hair to leaves. This will be seen to be true whether the osmotic or the imbibitional mechanism be assumed, since in any active cell there will be an equilibrium between the forces of the colloids present in the cell walls and the osmotic activities of the solutes of the cell sap. Since the actual concentration gradient observed does not conform to this requirement, and since positive exudation pressures are not thus explicable, these theories are incomplete.

If work is accomplished, the necessary energy must be furnished directly or indirectly by respiration processes and can be detected and estimated by them. The present study was undertaken with this in view.

The results obtained from a simultaneous measurement of water absorption and root respiration, as measured by the evolution of carbon dioxide

computed from the changes of pH in rain water, show a close correlation between the two processes. Even in the two experiments where the plants were exposed to light this relation was evident. When transpiration was increased by changing the air over the tops from a high to a low humidity, the rate of absorption increased and was accompanied or closely followed by a rise in the rate of carbon dioxide evolution.

When the evolution of carbon dioxide was followed in aerated distilled water a correlation similar to that observed in rain water appeared. In general this correlation was somewhat more perfect than that observed in rain water.

It is to be noted that, assuming the most intimate relation between root respiration and the absorption of water, a perfect correspondence between the rates would not be expected in experiments such as those here reported. This arises from the fact that some of the respiratory carbon dioxide is carried up in the transpiration stream and hence is not evolved (CERIGHELLI **2, 3, 4**). This may be considered as the explanation for the low rate of respiration accompanying high absorption at such points as the third hour of figure 4; and also for the failure, in cases like that shown in figure 6, of the respirational curve to follow the rise of absorption. Since the curves of carbon dioxide evolution and of oxygen consumption are parallel, any lack of agreement between the former phenomenon and the absorption of water is without significance in these experiments. This study gives no evidence of the transport of carbon dioxide or of any effects upon the evolution of the substance due to the photosynthetic activity of the leaves.

Computations from the results of experiments 1–6, in which the root systems were observed in rain water, show great variability and an average of 0.08 cc. (S.T.P.) carbon dioxide evolved per cubic centimeter of water absorbed. A similar average for plants observed with the root systems in distilled water is 2.6 cc. This difference is doubtless due to the buffering substances present in rain water and the latter value is to be regarded as the more accurate.

The consumption of oxygen offers a more reliable measurement of respirational activity, and in experiment 8 the absorption of 1 cc. of water required 3.9 cc. of oxygen and evolved 2.6 cc. of carbon dioxide, giving a respiratory quotient of 0.67. The average consumption of oxygen per cubic centimeter of water absorbed from distilled water is 2.4 cc. If we accept the value of 5.047 small calories per cubic centimeter of carbon dioxide, we have in experiment 8 an expenditure of approximately 13.1 calories per cubic centimeter absorption. If the consumption of oxygen be considered a more accurate measure of work, the result is approximately 19.7 calories.

Similar computations for plants observed with the root systems in Knop's solution give an oxygen consumption of 4.7 cc. per cubic centimeter

absorption. The average consumption of oxygen per volume absorption in distilled water is but 52 per cent. of that in Knop's solution. Computed on the basis of the average values obtained in distilled water, the oxygen consumption of the root systems in Knop's solution is 193 per cent. Comparing the calorific values per cubic centimeter absorption, we have 13 calories for distilled water and 23.7 for Knop's solution. Since the osmotic pressure of the Knop's solution used was 1.75 atmospheres, it appears that the energy required for the absorption of distilled water is equivalent to that expended against an osmotic pressure of $\frac{23.7 - 13}{1.75 \times 13}$, or 2.12 atmospheres.

In view of the fact that the resistance to flow through capillary tubes is a linear function of the rate, it might be expected that the energy requirement would vary directly with the rate of absorption. Computations show that the reverse relation holds in the present study, doubtless because the basal rate for the tissues comprises an increasing proportion of the whole, since at the lower absorption rates the time interval is increased.

It is to be noted that the respiration as here recorded was not anaerobic or modified by deficient aeration. This is obvious from the fact that the fluid around the roots was replaced at hourly intervals with aerated medium, and that the amount of dissolved oxygen contained in this was 1.4 cc. The observed absorption was never more than 0.5 cc. and usually was much less than this amount.

For corn seedlings in liquid cultures the correlation between the volume absorption and the respiration of the root indicates the expenditure of energy by these organs in the process of absorption. Further and more exact determinations of the energy values with plants under strictly controlled conditions should throw much light on the nature of the mechanism involved.

Summary

1. A method was developed for obtaining simultaneous measurements of root respiration and volume absorption of the roots.

2. The evolution of carbon dioxide was measured by change in the pH of the medium in the absence of buffers, and the absorption of oxygen was measured by the micro-Winkler method.

3. A correlation between the evolution of carbon dioxide, the absorption of oxygen, and the volume absorption of the root system of corn seedlings is shown to occur in rain water, distilled water, and in Knop's solution.

4. The energy as calculated from the respiratory exchange is 93 per cent. greater in Knop's solution with an osmotic pressure of 1.75 atmospheres than in water.

5. The absorption of water by corn roots is accompanied by the expenditure of energy.

The writer wishes to thank Dr. G. W. GOLDSMITH and others associated in the physiological work at the University of Texas for constructive suggestions and criticism.

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CARBOHYDRATE–NITROGEN AND BASE ELEMENT RELATIONSHIPS OF PEAS GROWN IN WATER CULTURE UNDER VARIOUS LIGHT EXPOSURES

ORMAN E. STREET

(WITH THREE FIGURES)

Introduction

The response of plants to the supply of various nutrient elements has been the subject of numerous investigations. The degree of response is largely governed by environmental factors, which may even nullify the effect of varying treatments. A dynamic material such as soil is apt to be much less responsive to treatment than is quartz sand, which in turn is a less responsive medium than are water cultures. It is often possible to obtain significant differences in growth and composition of plants in water cultures when the differences in nutrient supply are rather small.

In studies of carbohydrate-nitrogen relationships, the tendency has been to employ radical treatments, especially as regards the nitrogen supply, conclusions being advanced on comparisons between a very low and a very much higher level of nitrate nitrogen in the culture medium. In the present work, the nitrogen supply has been kept at the same order of magnitude in all cultures. The employment of differential water cultures of the same total osmotic concentration permitted this control.

Considerable differences in the organic composition of peas grown in water cultures were reported in 1928 by CLEMENTS (**7**). He concluded that under long light exposures nitrogen assimilation was influenced by the supply of potassium, and total carbohydrates were highest with high nitrogen treatments, while under shorter light exposures the greatest nitrogen assimilation was with an intermediate treatment, and the highest total carbohydrates with low nitrogen supply. The present work was started in the same laboratory with the purpose of testing these conclusions.

In view of the well established effect of light duration on growth and reproduction, the use of several light exposures was a necessary part of this study. As a continuation of this phase of the problem, it was decided to investigate the effect of light duration on the intake of base elements by the plant. Such problems as the effect of the season on the composition of the plant are perhaps related to light duration.

Literature review

The extensive literature on water cultures has been thoroughly reviewed by TOTTINGHAM (**25**) and others, and need not be enlarged here. The work of SHIVE (**24**) on salt combinations and of MCHARGUE (**19**), BRENCHLEY

(**6**), MAZÉ (**20**), and others on supplementary elements is also well reviewed. The exhaustive studies of GARNER and ALLARD (**9, 10, 11**) on light effects in plants have been extended by numerous workers, and the literature is well summarized by ARTHUR, GUTHRIE, and NEWELL (**3**). These investigators also attempted to control other climatic factors, such as moisture, temperature, and carbon dioxide concentration of the air, as well as the intensity, quality, and duration of light. All the studies support the generalization that an extension of light period brings about an increase in carbohydrate content and a decrease in nitrogen content.

The intake of base elements is apt to be a function of other factors than the supply of any one element, but under optimum conditions an intake proportionate to the supply will obtain. Among the fertilizer elements that cause a marked response in the plant, potassium is usually foremost. ANDERSON, SWANBACK, and STREET (**2**) reported a range of 1.16 to 6.78 per cent. K_2O on tobacco grown under various conditions. FONDER (**8**) has noted consistent differences in potash content of alfalfa on several soil types, while SAYRE (**23**), working with canning peas, obtained a range from 0.87 to 8.70 per cent. by varying the CaO/K_2O ratio in water cultures.

Responses to calcium and magnesium applications in soil are usually related to the relative abundance of these elements as compared with the potash supply, as has been shown by MORGAN (**21**) and HAAS (**12**). Application of lime materials containing both calcium and magnesium results in an increase of the magnesium content, but notably depresses the potassium and strongly reduces the calcium content as indicated by the work of ANDERSON *et al.* (**2**). The studies of MORGAN (**21**) also show that it is only when both potassium and magnesium are available in relatively small amounts that the plant is able to take up calcium freely.

References to the effect of light on the intake of bases are not numerous in the literature. BARTHOLOMEW and JANNSEN (**5**) conclude that potassium is taken up as freely at night as during the day. TYSON (**26**) grew sugar beets in compartments shaded to varying degrees, and found that the crude ash content of leaves increased with a decrease of light intensity, and that in general the same relation held for the three principal bases. NIGHTINGALE *et al.* (**22**) found that plants grown in a calcium deficient medium, when placed in darkness, showed the presence of "uncombined" calcium, *i.e.*, calcium that could be detected by the usual treatment with oxalic acid, and that the presence of calcium in this form permitted growth and perhaps protein elaboration.

Plan of experiment

The triangular system of TOTTINGHAM (**25**) and SHIVE (**24**), in which three-salt combinations having a total osmotic concentration of one atmos-

phere are used, was selected as a culture medium for these experiments. The same salt combination (KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$) as was used by CLEMENTS (7) was employed. Three-gallon crocks fitted with paraffined aluminum covers and capable of growing 40 plants were used, and duplicate cultures of each treatment grown. The cultures listed in table I were chosen as being representative of different portions of the triangle, as well as giving several combinations of potassium and nitrogen, under the conditions recommended by LIVINGSTON *et al.* (17).

TABLE I

DIFFERENTIAL CULTURE SOLUTIONS, TYPE 1
OSMOTIC CONCENTRATION, 1 ATMOSPHERE

SOLUTION NUMBER	MOLECULAR PROPORTIONS			PARTIAL VOLUME MOLECULAR PROPORTIONS		
	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
1–1–6	1	1	6	0.0027	0.0027	0.0161
1–6–1	1	6	1	0.0020	0.0122	0.0020
2–2–4	2	2	4	0.0049	0.0049	0.0099
3–3–2	3	3	2	0.0068	0.0068	0.0045
5–1–2	5	1	2	0.0123	0.0024	0.0049

In addition to the salts employed (table I), small amounts of iron, manganese, and boron were added to the culture solutions. Preliminary tests showed that 2.3–4.0 p.p.m. iron in ferric tartrate, 0.66–2.0 p.p.m. boron in boric acid, and 0.55–2.0 p.p.m. manganese in manganous chloride were the optima, and the lower amounts were used in subsequent experiments.

The culture solutions were changed once a week. Transpiration losses were made up by the addition of distilled water during the latter part of the growing period. The cultures were aerated for a period of two hours each day.

Light exposures of 10, 13, and 17 hours were chosen as representing the range of light period under which normal growth might be expected. The longer periods were in part artificial light, the ten cultures of each group being arranged under a battery of lights suspended 5 feet above the greenhouse benches and supplying 2000 watt at 110 volts. Measurements of light intensity were not made, as the duration of light has been found by GARNER *et al.* (11) to be the most significant factor, provided the intensity is not too low. However, comparison of illumination per unit area (as used in these experiments) with that employed by ARTHUR *et al.* (3) would indicate that the intensity was high. No water screens between the lights

and the plants were available but the heating effect was not noticeable. As the plants grew taller, the lights were raised.

The 10-hour plants received only solar light, altered, of course, by the glass of the greenhouse. These cultures were grown in large wooden boxes which were fitted with light-tight covers and ventilated by a shutter arrangement similar to that employed in photographic dark rooms. The period of illumination of these cultures was 8 A. M. to 6 P. M.

The 13-hour plants were protected from the artificial light source of the 17-hour plants by curtains of fine black cambric cloth. These curtains were drawn around the bench at the end of the 13-hour period and withdrawn early in the morning.

Nott's Excelsior field peas, obtained from the Michigan State Farm Bureau, Lansing, Michigan, were used throughout the experiments. They were found to be an excellent type for this work, having a sturdy growth habit and flowering freely. Before being placed in the germinator the seeds were sterilized in 1–250 formalin for 20 minutes, washed in tap water, and soaked for a few hours. They were then placed between layers of moist paper toweling in a large galvanized iron pan. After about three days a uniform lot of the more vigorous seedlings with roots measuring one inch in length was selected for the experiment. These selected seedlings were fitted into perforated corks each of which held five plants. The three-gallon culture jars were fitted with perforated aluminum covers, each of which held eight corks, making a total of 40 plants per culture.

In the first two series the seedlings which were damaged in handling were replaced during the first few days of the experiment. In the final series several extra corks were prepared and allowed to remain in contact with tap water until the experiment had progressed about two days, when replacement of corks not having a complete stand was made. By the latter technique a somewhat more uniform stand was obtained.

The chemical analyses were made on the tops and pods only, as follows: nitrate nitrogen, total nitrogen not including nitrates, simple sugars, sucrose, starch and hemicellulose, crude ash, potassium, calcium, and magnesium. Physical measurements included top length in centimeters, green weight of tops (and pods when found), oven-dry weight of tops, roots, and pods.

Chemical methods

In order to obtain comparable results, the chemical methods for carbohydrates and total nitrogen not including nitrates (organic nitrogen) were the same as those reported by CLEMENTS (7). All samples for the determination of carbohydrates were preserved in alcohol to which a small quantity of ammonium hydroxide was added to neutralize plant acids.

The DEVARDA method for nitrate nitrogen was tried but gave such

unreliable results that it was soon discarded. The impossibility of preventing breakdown of the simpler nitrogenous compounds such as amino acids and amides, and the consequent high results, render the method worthless for plant material. The modification of the GILBERT method by HOLTZ and LARSON (**16**) gave very good results.

Crude ash and calcium were determined by the official methods of the A.O.A.C. (**4**). Magnesium was determined by the volumetric method of HANDY (**13**). Potassium determinations of the first series were made by the sodium cobalti-nitrite method of ADIE and WOOD (**1**). A comparison of this method with the official chloroplatinate procedure showed that the former method did not give consistent results, most of the percentages being too high. Series I was therefore repeated and all potassium figures reported are by the official method. All figures reported are on oven-dry-weight basis.

Experimental results

Series I was started March 13 and ran until May 14, 1928, a period of nine weeks. Blossoms appeared under 17-hour light three weeks after transplanting the seedlings. Both of the longer light periods produced pods abundantly, and at the time of harvest the pods were beginning to ripen. The short-light plants blossomed toward the end of the period, but developed very few pods. Control of the temperature in the greenhouse was not difficult during these months, and cultural conditions were generally satisfactory. It was necessary to use lemon oil spray to control red spiders in the greenhouse and to give the cyanide treatment for white flies and aphids. The plants were not at any time seriously affected by these insects.

Series II ran from June 13 to July 31, 1928, a period of seven weeks. Owing to the higher air temperature in the greenhouse at this time, the plants matured more rapidly, and the uniformity of plants in a single culture was not so satisfactory. The light intensity was also higher at this later time, and this apparently had some effect on the growth in height and the carbohydrate content of the plants.

In series III it was decided to limit the experiment to 10-hour and 13-hour light periods, to have treatments in triplicate, and to terminate the experiment when the plants were vegetatively developed but had not set fruit. The cultures were started May 14 and ran until June 12, 1929, a period of four weeks. At this season of the year the days were long enough to omit the use of artificial light for any part of the 13-hour period.

PHYSICAL MEASUREMENTS

Physical measurements for all series are presented in table II. In order to establish the relative value of the various treatments in dry weight

production, the total dry weight per plant, exclusive of pods, was reduced to a ratio, taking the best treatment in each group as unity. These ratios are given in the last column of the table.

The average top length was usually greatest in the treatments supplying the highest proportion of nitrates. The dry weight production was highest under similar conditions, solution 1–6–1 leading in six out of eight groups and solution 3–3–2 being second best in the same number of cases. A weighted average of these ratios shows that the latter solution was 98.9 per cent. as efficient as the former in dry weight production. Solution 5–1–2, high in potassium, produced 86.9 per cent. of the dry weight of the 1–6–1 treatment; while 1–1–6 and 2–2–4, both high in magnesium, each produced 83.2 per cent.

Carbohydrate-nitrogen relationships

Results of carbohydrate analysis of series I, tops, are shown in table III. The term tops as used in this paper includes all the aerial parts of the plant except pods. It was not possible to separate these parts and secure samples large enough for analysis. All carbohydrates are computed to d-glucose using Munson and Walker's table (**4**). The figures presented are the average of the duplicate cultures, with duplicate determinations of each sample. Thus each entry represents the average analysis of a group of 80 plants.

As an aid to interpretation of the results, the ratio system was again employed. Ratios were computed upon the total sugars (sum of the simple sugars and sucrose), total non-sugars (sum of starch and hemicellulose), and total carbohydrates.

In this series the culture solutions highest in magnesium sulphate were the most efficient in promoting the elaboration of sugars. This was true in all light exposures, the only discrepancy being in the 2–2–4 treatment in the 13-hour light period. Solution 5–1–2, high in potassium, was not quite so efficient, while the high nitrate solution was only 70 per cent. as high in total sugars as the 1–1–6 treatment.

In the formation of more stable carbohydrate compounds, the most efficient solution was found to be the high potassium treatment. Although it did not consistently lead in all light exposures, the average of the ratios placed it in the fore. The magnesium treatments were consistently good, while the high nitrate treatment was again the least efficient.

In a study of the total carbohydrates, the most striking difference is the low total content of the plants in solution 1–6–1. Because of the superiority of the magnesium treatments in the production of sugars and the potassium treatments in the transformation of sugars into non-sugars, the two groups are about equally effective in maintaining a high total carbohydrate level.

It is apparent from the data that the duration of light was a significant factor in determining the general level of carbohydrate storage in the plant.

Table III also presents the results of determinations of organic and

TABLE II

PHYSICAL MEASUREMENTS OF PEAS GROWN IN WATER CULTURE

TREATMENT*	AVERAGE TOP LENGTH	AVERAGE DRY WEIGHT PER PLANT			DRY WEIGHT† (RATIO)
		TOPS	ROOTS	PODS	
		SERIES I *17 hours light*			
	cm.	*gm.*	*gm.*	*gm.*	
1–1–6	52.5	0.8363	0.1860	0.6390	0.80
1–6–1	52.5	1.0865	0.2774	0.7719	1.00
5–1–2	47.0	0.7649	0.1904	0.5500	0.70
3–3–2	51.6	0.9184	0.2755	0.6210	0.87
2–2–4	45.5	0.6216	0.1559	0.5206	0.57
		13 hours light			
1–1–6	50.8	0.7886	0.1535	0.4051	0.70
1–6–1	50.6	0.8404	0.1817	0.3264	0.76
5–1–2	49.9	0.9025	0.2349	0.3704	0.85
3–3–2	53.9	0.9300	0.3826	0.4315	0.98
2–2–4	50.8	1.0812	0.2527	0.5277	1.00
		10 hours light			
1–1–6	39.0	0.4537	0.1058		0.67
1–6–1	45.6	0.7007	0.1361		1.00
5–1–2	33.5	0.4195	0.1066		0.62
3–3–2	40.8	0.4984	0.1429		0.76
2–2–4	41.5	0.4402	0.1506		0.71
		SERIES II *17 hours light*			
1–1–6	36.5	0.6640	0.0962	0.1854	0.71
1–6–1	38.8	0.9160	0.1456	0.2703	1.00
5–1–2	36.3	0.7541	0.1328	0.2105	0.83
3–3–2	39.7	0.8785	0.1297	0.1171	0.94
2–2–4	34.4	0.7158	0.0979	0.1248	0.76
		13 hours light			
1–1–6	33.8	0.5183	0.0824	0.0578	0.75
1–6–1	42.0	0.7092	0.0896	0.1000	1.00
5–1–2	39.0	0.6612	0.1152	0.0567	0.97
3–3–2	36.9	0.6142	0.0998	0.1153	0.89
2–2–4	31.0	0.4919	0.0792	0.0828	0.77
		10 hours light			
1–1–6	21.8	0.2759	0.0337		0.81
1–6–1	24.2	0.2296	0.0400		0.70
5–1–2	24.3	0.2604	0.0527		0.79
3–3–2	25.3	0.3313	0.0624		1.00
2–2–4	23.5	0.2233	0.0467		0.68

TABLE II—(*Continued*)

Treatment*	Average top length	Average dry weight per plant			Dry weight† (ratio)
		Tops	Roots	Pods	
	Series III *13 hours light*				
1–1–6	24.6	0.3386	0.0830		0.89
1–6–1	32.4	0.3832	0.1012		1.00
5–1–2	30.0	0.3275	0.0936		0.86
3–3–2	30.6	0.3526	0.0939		0.92
2–2–4	25.4	0.3327	0.0785		0.84
	10 hours light				
1–1–6	24.3	0.2609	0.0604		0.92
1–6–1	25.7	0.2758	0.0722		1.00
5–1–2	20.3	0.2366	0.0624		0.86
3–3–2	22.8	0.2760	0.0657		0.98
2–2–4	24.7	0.2500	0.0614		0.89

* Culture solution, Shive's 1.00 atmosphere: 1st figure $KH_2(PO_4)$, 2nd figure $Ca(NO_3)_2$, 3rd figure $MgSO_4$.

† Dry weights of tops and roots, but not of pods, were computed to a ratio by giving the highest weight in the group the value of 1.00 and reducing others to decimal parts of this value.

nitrate nitrogen in this series. The nitrate nitrogen is at a much lower level than Clements (7) found in his work, resulting entirely from the difference in analytical methods. The only value of the data on nitrate nitrogen is to indicate the reserve supply in the plant, and this is naturally much higher with a short light exposure.

The content of organic nitrogen was entirely inconsistent with the supply of nitrogen in the medium. Here again the light duration determined the level of assimilation. No evidence of a correlation between potassium supply and organic nitrogen synthesis was found.

The pods produced under 17-hour and 13-hour light exposures in series I were also analyzed. The data were of interest only because they showed the high level of carbohydrate content in the partly mature fruit (average of 33.64 per cent. in long light and 29.77 per cent. in intermediate light), but they failed to disclose any consistent relationship with treatment. The organic nitrogen content (average of 3.25 per cent. in long light and 3.22 per cent. in intermediate light) failed to show even the effect of light exposure.

Series II was a repetition of series I, so far as the general plan of the experiment was concerned. Starch and hemicellulose were determined separately, but are reported together as acid-hydrolyzable carbohydrates. The average content of starch was very low, and did not justify separate discussion. The results are given in table IV.

TABLE III

CONTENT OF CARBOHYDRATE FRACTIONS AND NITROGEN IN TOPS
SERIES I

TREATMENT	SIMPLE SUGARS	SUCROSE	TOTAL SUGARS (RATIO)	STARCH	HEMICELLULOSE	NON-SUGARS (RATIO)	TOTAL CARBOHYDRATES		ORGANIC NITROGEN	NITRATE NITROGEN
							PERCENTAGE	RATIO		
	17 hours light									
	%	%		%	%		%		%	%
1–1–6	5.31	3.40	0.99	2.99	3.03	1.00	14.73	0.94	3.17	0.150
1–6–1	3.12	1.77	0.56	1.85	2.62	0.74	9.36	0.59	3.39	0.186
5–1–2	3.31	4.23	0.89	1.74	4.17	0.98	13.45	0.85	3.31	0.105
3–3–2	2.77	5.40	0.93	0.64	4.65	0.87	13.48	0.86	3.42	0.222
2–2–4	4.93	3.80	1.00	1.22	4.80	1.00	15.75	1.00	3.27	0.163
	13 hours light									
1–1–6	3.74	3.49	1.00	2.69	2.70	0.85	12.62	0.99	3.51	0.192
1–6–1	2.69	2.82	0.75	1.77	3.09	0.76	10.37	0.81	3.80	0.745
5–1–2	3.05	3.35	0.88	3.50	2.86	1.00	12.76	1.00	3.06	0.072
3–3–2	3.88	3.27	0.99	2.34	1.94	0.67	11.43	0.89	3.66	0.655
2–2–4	3.41	2.51	0.82	2.55	2.54	0.80	11.01	0.86	3.21	0.368
	10 hours light									
1–1–6	2.50	2.74	0.95	0.84	2.73	0.81	8.81	0.89	4.56	0.268
1–6–1	2.30	2.09	0.79	0.93	2.90	0.87	8.22	0.83	4.67	0.698
5–1–2	2.91	1.87	0.86	0.97	3.28	0.97	9.03	0.91	4.68	0.388
3–3–2	1.22	3.90	0.93	0.73	3.53	0.97	9.38	0.95	4.74	0.400
2–2–4	2.76	2.76	1.00	0.83	3.55	1.00	9.90	1.00	4.82	0.505

TABLE IV

CONTENT OF CARBOHYDRATE FRACTIONS AND NITROGEN IN TOPS
SERIES II

TREATMENT	SIMPLE SUGARS	SUCROSE	TOTAL SUGARS (RATIO)	ACID-HYDROLYZABLE CARBOHYDRATES		TOTAL CARBOHYDRATES		ORGANIC NITROGEN	NITRATE NITROGEN
				PERCENTAGE	RATIO	PERCENTAGE	RATIO		
	17 hours light								
	%	%		%		%		%	%
1–1–6	10.34	4.00	1.00	7.81	1.00	22.15	1.00	3.071	0.006
1–6–1	10.75	1.91	0.88	6.78	0.87	19.44	0.88	3.487	0.188
5–1–2	5.73	2.19	0.55	7.52	0.96	15.44	0.70	3.241	0.006
3–3–2	6.56	2.30	0.62	6.50	0.83	15.36	0.69	3.527	0.215
2–2–4	9.15	2.21	0.79	6.81	0.87	18.17	0.82	3.402	0.003
	13 hours light								
1–1–6	8.23	5.40	1.00	5.67	0.45	19.30	1.00	3.967	0.004
1–6–1	1.66	4.19	0.43	12.74	1.00	18.59	0.96	3.793	0.119
5–1–2	3.88	2.76	0.49	11.33	0.88	17.97	0.93	3.770	0.025
3–3–2	5.55	2.20	0.57	10.21	0.80	17.96	0.93	3.712	0.005
2–2–4	5.95	4.40	0.76	7.10	0.56	17.45	0.90	3.961	0.005
	10 hours light								
1–1–6	3.29	1.39	0.91	5.83	0.63	10.51	0.80	4.843	0.211
1–6–1	4.49	0.57	0.98	7.86	0.85	12.92	0.97	4.711	0.215
5–1–2	3.45	0.57	0.78	9.20	1.00	13.22	1.00	4.657	0.381
3–3–2	4.20	0.94	1.00	6.85	0.74	11.99	0.90	4.622	0.437
2–2–4	3.59	0.92	0.88	5.15	0.56	9.66	0.73	4.653	0.400

The general level of carbohydrate accumulation in this series was above that of series I. As mentioned previously, perhaps this was a response to the higher air temperatures in the greenhouse. In this series there was not a clear distinction between the levels of carbohydrate accumulation in long and in intermediate light exposures, the temperature factor apparently overshadowing the light effect.

The effect of high magnesium on the amount of sugars found was similar to that in series I, although only treatment 1–1–6 was able to maintain a consistently high level, treatments 2–2–4 and 3–3–2, the latter in particular, being somewhat erratic except in short light. In this series the sugar content of the high potassium culture was lower than in the high calcium treatment. The high level of non-sugars in the potassium cultures is an evident corollary of the low sugar content, as rapid transformation must have occurred.

The lowest content of polysaccharides was found in the highest magnesium treatment, if the ratios of the three light treatments are averaged. It is hardly safe to conclude that this is a fixed relation, however, as the cultures varied widely in this respect.

No one treatment was greatly superior in total carbohydrate accumulation, although the high magnesium treatment led in this respect in long and in intermediate light. The very high percentages found with all treatments tended to mask any effects due to nutrients.

The nitrogen data showed almost no differences between nutrient treatments at any light duration. The light effect, *per se*, was again distinctly shown.

In the third series it was decided to grow the plants only until the first blossoms appeared, to determine whether the differences could be observed before the plants were mature. It was also decided to limit the light durations to 10 and 13 hours, as the greater differences had been found between these durations. Triplicate cultures, involving the growth of 120 plants under each condition, were used, instead of the duplicate cultures in the previous series.

Analytical results of this series are presented in table V. In this table the column "Acid-hydrolyzable carbohydrates" represents only one analytical step, no attempt being made to separate starch from hemicellulose.

The level of carbohydrate accumulation at this stage of the plant's growth was distinctly lower in the 13-hour exposure than was the case when the plants were grown to maturity. With 10-hour exposure, the final level was more nearly like that found in series I.

The highest level of sugar accumulation was found with treatment 2–2–4, as an average of both light durations. Treatment 1–1–6 was unaccountably low in these fractions, while the high potassium treatment was

TABLE V

CONTENT OF CARBOHYDRATE FRACTIONS AND NITROGEN IN TOPS
SERIES III

TREATMENT	SIMPLE SUGARS	SUCROSE	TOTAL SUGARS (RATIO)	ACID-HYDROLYZABLE CARBOHYDRATES		TOTAL CARBOHYDRATES		ORGANIC NITROGEN	NITRATE NITROGEN
				PERCENTAGE	RATIO	PERCENTAGE	RATIO		
	10 hours light								
	%	%		%		%		%	%
1–1–6	2.07	0.45	0.66	6.09	0.91	8.61	0.81	4.511	0.072
1–6–1	2.42	0.25	0.70	5.48	0.82	8.15	0.77	4.459	0.330
5–1–2	2.59	0.37	0.77	5.70	0.85	8.66	0.82	4.414	0.150
3–3–2	3.61	0.23	1.00	6.69	1.00	10.53	1.00	4.345	0.136
2–2–4	2.87	0.16	0.79	6.25	0.93	9.28	0.88	4.259	0.030
	13 hours light								
1–1–6	2.04	0.66	0.77	4.59	0.73	7.29	0.76	5.203	0.358
1–6–1	2.21	0.39	0.74	5.57	0.89	8.17	0.85	5.064	0.427
5–1–2	2.63	0.71	0.98	6.23	1.00	9.57	1.00	4.842	0.188
3–3–2	2.32	0.08	0.69	4.44	0.71	6.84	0.71	4.861	0.299
2–2–4	3.23	0.37	1.00	5.81	0.93	9.41	0.98	4.605	0.261

very good in the short light. The last treatment was again conducive to non-sugar formation in this series, although not superior to treatment 2–2–4. These two treatments were also best in total carbohydrates.

The content of organic nitrogen was the highest in each case in the high magnesium treatment. The differences were only a few hundredths of 1 per cent. and are hardly worthy of mention. The light effect on the level of protein elaboration was noticeable even at this stage of growth.

A general summation of the results of these studies, and a consideration of average ratios, show that the culture solutions might be ranked as follows with respect to production of sugars: 1–1–6, 2–2–4, 3–3–2, 5–1–2, 1–6–1. This indicates that the proportion of magnesium sulphate in the culture solution, indicated by the third figure in each group, bears considerable relation to sugar content. The treatment high in calcium nitrate was rather consistently low in these fractions, while high potassium occupied an intermediate position.

With respect to polysaccharides, the following ranking seems justified: 5–1–2, 1–6–1, 2–2–4, 3–3–2, 1–1–6. Only in the case of the high potassium treatment was there a sharp distinction, as it was greatly superior in nearly all cases.

Total carbohydrates, as mentioned previously, tended to be at about the same level whether the solution was high in potassium or in magnesium. They were clearly lower in the high calcium treatment, excepting in series II in which an excessively high level prevailed under all conditions.

Base element relationships

The material for this study was the oven-dried samples of the tops. No analyses of pods were made. Data on crude ash for all series are given in table VI.

The average amount of crude ash was greater under 10-hour light duration than under the 13-hour in every case. Long light duration was not consistent, being the lowest in series I and the highest in series II.

The effect of nutrient materials on the crude ash was clearly shown. In every case except one, the lowest figure was found with the high magnesium treatment, 1–1–6. The highest percentage of crude ash was noted with the high potassium or high calcium treatment, or a combination of the two, in all groups. This is in accordance with the relative percentages of the three elements commonly found in plants.

Table VII presents the analyses of the three dominant bases in the tops for all series.

The data for series I are also presented in graphic form in figure 1. In this graph the increments of potassium (represented by the first of the three figures) increase from left to right. The increments of calcium (rep-

TABLE VI

PERCENTAGE OF CRUDE ASH IN PEA PLANTS

Treatment*	Percentage crude ash		
	Series I	Series II	Series III
	17 hours light		
	%	%	%
1–1–6	12.71	15.35	.....
1–6–1	12.76	15.91	.....
5–1–2	15.47	18.05	.....
3–3–2	14.59	17.37	.....
2–2–4	14.88	16.85	.....
	13 hours light		
1–1–6	13.41	12.80	11.80
1–6–1	15.48	15.00	16.43
5–1–2	15.21	15.85	17.08
3–3–2	16.43	14.12	14.09
2–2–4	16.55	14.71	13.20
	10 hours light		
1–1–6	14.95	14.96	15.31
1–6–1	16.32	16.71	16.09
5–1–2	16.10	18.22	14.38
3–3–2	15.05	15.77	15.12
2–2–4	15.97	14.97	15.25

* Culture solution, Shive's 1.00 atmosphere: 1st figure KH_2PO_4, 2nd figure $Ca(NO_3)_2$, 3rd figure $MgSO_4$.

resented by the second figure) and of magnesium (represented by the third figure) decrease from left to right. To illustrate more clearly the repressive effect of potassium or the other two bases, the treatment 5–1–2 was placed at the right of all sections of the graph.

Considering first the correlation between the supply of any one base in the culture solution and the percentage in the plants produced in that solution, it can be seen that a very good agreement existed. The only exceptions were the potassium content of treatment 3–3–2 grown in 13-hour light duration and the higher magnesium increments with the same exposure.

Evidence on the mutually repressive effect of the bases was clearly apparent. A high proportion of potassium in the culture solution repressed the calcium intake under all conditions to a greater extent than did a large amount of magnesium. Similarly solution 1–6–1, high in calcium, invariably produced plants with a higher magnesium content than did solution 5–1–2, high in potassium, although the latter supplied twice as much mag-

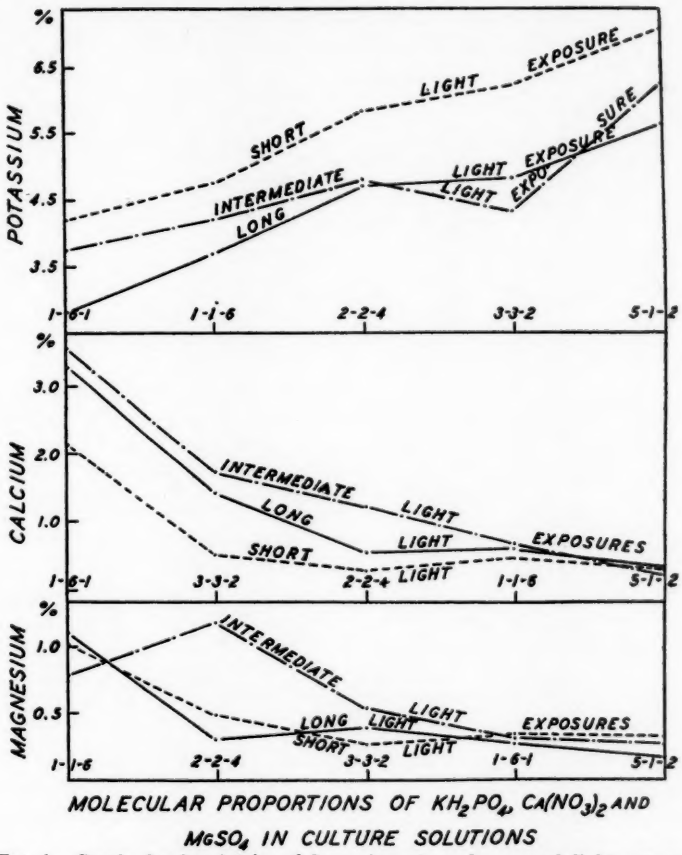

FIG. 1. Graph showing intake of base elements under several light exposures in series I.

nesium. Calcium showed a greater repressive effect than magnesium on the intake of potassium. In all cases solution 1–6–1 produced plants lower in potassium content than did solution 1–1–6.

The most marked effect of the duration of light on the absorption of bases was upon the intake of potassium. With the 10-hour light period the level of potassium intake was significantly higher throughout the range of cultural treatment. An intermediate photoperiod gave higher percentages of this element than long light, with the exception of one treatment. Between the extreme treatments an average difference of over 1 per cent. in potassium content was found.

TABLE VII

CONTENT OF POTASSIUM, CALCIUM, AND MAGNESIUM IN TOPS OF PEAS

TREATMENT	PERCENTAGE ON OVEN-DRY BASIS								
	SERIES I			SERIES II			SERIES III		
	K	Ca	Mg	K	Ca	Mg	K	Ca	Mg
	17 hours light								
	%	%	%	%	%	%	%	%	%
1–1–6	3.64	0.58	1.13	4.26	0.36	1.50	.....	.....	.....
1–6–1	2.75	3.32	0.27	4.01	2.81	0.40	.....	.....	.....
5–1–2	5.64	0.27	0.15	6.50	0.24	0.27	.....	.....	.....
3–3–2	4.81	1.45	0.41	5.47	0.77	0.90	.....	.....	.....
2–2–4	4.69	0.54	0.30	5.16	1.40	1.37	.....	.....	.....
	13 hours light								
1–1–6	4.20	0.61	0.80	5.05	0.29	0.92	5.01	0.35	0.99
1–6–1	3.67	3.61	0.33	4.75	2.16	0.31	5.14	1.26	0.35
5–1–2	6.38	0.19	0.27	7.05	0.16	0.19	6.38	0.20	0.30
3–3–2	4.37	1.70	0.53	6.37	1.09	0.26	5.86	0.28	0.36
2–2–4	4.83	1.25	1.19	5.48	0.63	0.43	4.95	0.33	0.64
	10 hours light								
1–1–6	4.76	0.47	1.00	5.90	0.26	0.60	6.54	0.28	0.56
1–6–1	4.20	2.18	0.33	5.76	1.36	0.30	6.29	1.01	0.32
5–1–2	7.10	0.22	0.32	8.10	0.16	0.23	6.81	0.20	0.27
3–3–2	6.27	0.51	0.28	6.96	0.37	0.35	5.41	0.24	0.29
2–2–4	5.87	0.29	0.50	6.51	0.28	0.52	5.77	0.28	0.46

Calcium content in reference to duration of light showed the 13-hour day length able to maintain the highest level. Short light duration was featured by the lowest relative level of calcium absorption, and except in the highest concentration by a rather low absolute content.

With respect to magnesium the intake was not consistent. Intermediate light duration permitted a greater intake than long light in all but one treatment. Under 10-hour day length the range of magnesium content was narrower, the low magnesium cultures having a slightly higher intake and the high concentrations being somewhat less than under greater light durations.

The data for series II also are presented in table VII, and shown graphically in figure 2. The correlation between the supply present and the absorption was considerably better in most respects. The calcium content of the plants grown in solution 3–3–2 under maximum light exposure was lower than in solution 2–2–4, but this was the only inconsistency in this respect in the series.

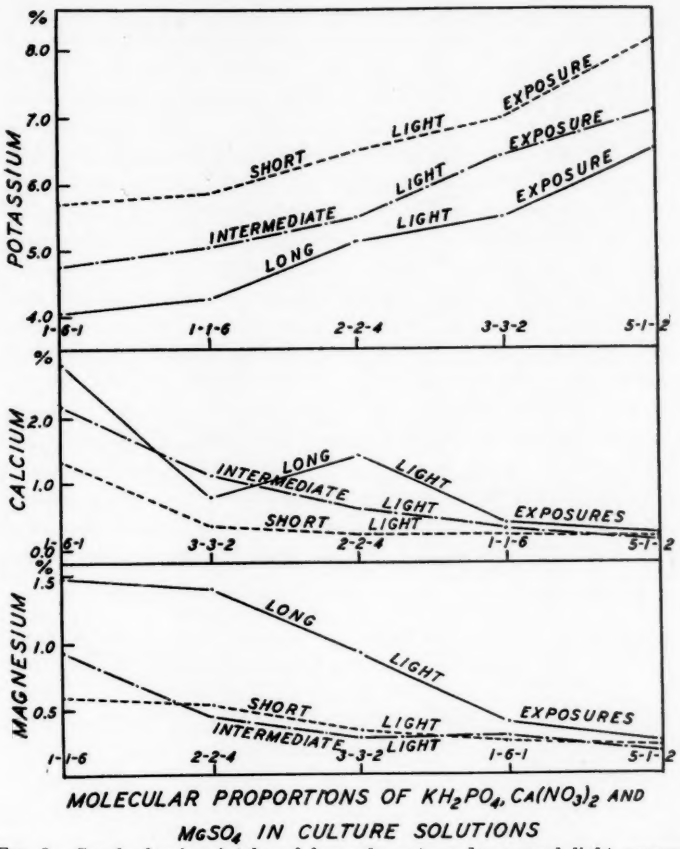

FIG. 2. Graph showing intake of base elements under several light exposures in series II.

The various repressive effects noted in series I were again evident in this series. The high potassium culture, 5–1–2, was particularly repressive to calcium and magnesium absorption in all cases.

Very definite differences in potassium absorption with respect to light duration were found. Plants subjected to a 10-hour exposure were again the highest in potassium content, while intermediate exposure was clearly more conducive to potassium intake than long exposure. As might be expected in view of the antagonism of potassium and calcium, the short exposure plants had the lowest level of calcium intake. The differences between long and intermediate light periods were not so conclusive, but favored the former in all but one treatment.

Magnesium was considerably higher under long light exposure than in series I, and definitely greater than in the shorter light exposures of this series.

Because the plants in the third series were grown for a period of only four weeks, the results as shown in table VII and figure 3 are not entirely comparable with those of the preceding series. The response to high or to low supply of the different elements was not so great, consequently the range of percentages was much reduced. A fairly high level of potassium content was reached but calcium and magnesium were very low. The exceedingly low magnesium content of the young leaves was in agreement with results of LUTMAN and WALBRIDGE (**18**) on potatoes analyzed at several stages of growth. Potassium was somewhat antagonistic to the other bases, but the relatively greater repressive effect of calcium toward potassium had not begun to be evident.

The effect of light duration was more clearly shown in the content of calcium and magnesium than in that of potassium. Both bases were absorbed in direct relation to the light period. Apparently the absorption of these elements was rather regular during the early stages of growth, and no great accumulation took place in any culture. On the other hand, the potassium was not taken up in accordance with the supply, at least in the

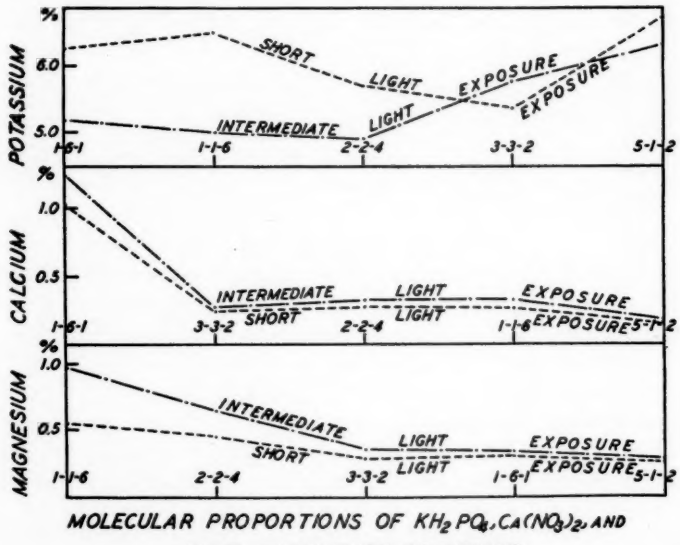

FIG. 3. Graph showing intake of base elements under several light exposures in series III.

lower increments. Only in the solution containing the largest amount of potassium was there an abundant intake. In the other cultures, one part of potash in the solution seemed to permit as great an intake as three. This would indicate that a lower concentration of potash is adequate for young plants.

Considering the evidence of the first two series, it seems conclusive that potassium was most abundant in plants grown to maturity under a 10-hour light exposure. As the light period was lengthened the level of potassium intake dropped. In young plants these phenomena were not so well defined.

On the other hand, calcium was most abundant under longer light periods, with 13-hour and 17-hour durations about equally efficient in promoting absorption of this element. Magnesium was similarly affected by the photoperiod.

In attempting to explain the results of these experiments, it is well to remember that on these same plants, determinations of organic and total nitrogen showed that the shorter the light period, the higher the percentage of nitrogen. This was in accordance with the findings of many other investigators. Moreover the length of day was practically the only controlling factor, as the plants were able to get an adequate supply of nitrogen from the lowest increments, and therefore failed to respond to increased amounts.

Thus it may be assumed that, in a series of culture solutions having the same osmotic concentration but varying proportions of the three salts, all the plants in the series would have an equal supply of base elements. It is generally agreed that an acid-base equilibrium exists in plants. If light conditions were such as to permit a heavy intake of nitrates, an equivalent amount of one or more bases must also enter the plant. In these experiments, potassium was apparently available in the greatest relative amount; hence its high level in the short-light plants which were all equally high in organic nitrogen. It is also significant that it was with short light only that nitrate nitrogen was present to any amount in these plants. In experiments on the same general theme, HIBBARD (**14**) found an accumulation of nitrogen in the plants grown in short light, proportional to the amount of calcium. In this case calcium was apparently the most abundant cation, although no analyses of plants are presented to show whether the plants had absorbed more calcium.

The absorption of calcium, and to a large extent of magnesium, seems to bear a reciprocal relation to that of potassium. The statements of HOAGLAND and MARTIN (**15**) in reference to toxicity of salts under various climatic conditions are of interest in connection with these studies. They found that solutions which stimulated growth under certain conditions might produce marked inhibition of growth at a different time of year.

Summary and conclusions

1. Nott's Excelsior field peas were grown in selected water cultures, SHIVE's 1.00 atmosphere, type 1. Analyses of tops for carbohydrate fractions, organic and nitrate nitrogen, crude ash, potassium, calcium, and magnesium are reported. The effect of light duration on the relative amounts of these plant constituents was studied. Physical measurements are given in all cases.

2. Results of physical measurements indicate that the highest average top length, dry weight of tops, and dry weight of entire plants exclusive of fruit were found with solutions high in calcium nitrate. With respect to the last measurements, a high potassium treatment was more effective than were high magnesium treatments.

3. Highest content of total sugars (simple sugars and sucrose) was found with solutions high in magnesium. Polysaccharides (starch and hemicellulose) was markedly high with an abundant supply of potassium.

4. Total carbohydrates were maintained at a fairly high level by all treatments except the one high in calcium nitrate. This is in direct contrast to the findings of CLEMENTS (7) for long light conditions, but in partial agreement with his analyses for shorter light periods, and in good agreement with earlier findings.

5. No consistent difference in content of organic nitrogen due to difference in culture solution was found in any case. This finding also contrasts with the results reported by CLEMENTS, who found that a high potassium supply was correlated with high total nitrogen in the plant.

6. Crude ash was higher in plants grown in a 10-hour than in a 13-hour light period, but not consistently higher than in plants grown in a 17-hour duration. A culture solution high in magnesium resulted in the lowest crude ash in all but one case. High potassium or high calcium solutions grew plants which were the highest in crude ash.

7. Light exposures of 10 hours daily produced plants high in potassium and low in calcium and magnesium.

8. Light exposures of 13 hours produced plants lower in potash and higher in calcium and magnesium than 10-hour plants. This exposure in series I had the highest level of calcium intake.

9. Light exposures of 17 hours resulted in plants markedly low in potash and usually the highest in calcium and magnesium.

10. The correlation between the relative supply of any one element in a group of cultures and the relative intake of that element by the plants grown in these cultures was clearly marked.

11. The mutually repressive effect of the three bases was demonstrated. Potassium was so strongly repressive as to overshadow other factors when

the supply of this element was high and the supply of the other bases rather low.

The writer is indebted to Dr. R. P. HIBBARD for guidance during the course of the experiments, to Dr. H. F. CLEMENTS for suggestions in planning the work, and to Dr. P. J. ANDERSON for permission to complete the necessary analytical work.

CONNECTICUT AGRICULTURAL EXPERIMENT STATION
WINDSOR, CONNECTICUT

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APPLICATION OF CALORIMETRIC METHODS TO ECOLOGICAL RESEARCH

FRANCES L. LONG

(WITH TWO FIGURES)

The method of determining the heat of combustion by means of an oxygen bomb calorimeter has been adapted to plant studies of energy relations. This is based on standard chemical and physical practise, so that results obtained by one worker can be compared with those obtained by another. In general, it is the method by means of which heating values for coal and other fuels are obtained; the procedure that is used for determinations of caloric values for fats, proteins, and sugars upon which diets are based.

Prompt drying of plant material at moderate temperatures immediately after collecting is essential to accuracy, owing to the destructive effects of respiration and enzyme action. This demands an oven with considerable capacity, special ventilation, and uniformity of temperature throughout. An oven to meet these requirements was built for this work and is described in the paper by MARTIN.[1] The thermo-regulator is set for 75° C., since this temperature, aided by air currents, affords the most favorable condition for drying plant material of the type used here, with the minimum risk of destructive effects during the process. After drying, the material is ground fine, thoroughly mixed, and then made into pellets by means of a pellet press. To prevent these pellets from absorbing atmospheric moisture, which makes them so large and loose that they may fall from the capsule, they must be kept in a desiccator until required for use.

In calorimeters of the usual type having outside walls of bakelite or other insulating material, there is a heat interchange between the interior of the instrument and the surrounding air. Although the best insulation possible is used, it is still necessary to take heat losses into consideration and to make corrections for radiation to secure accurate results. The calorimeter used for the present studies was so constructed as to secure adiabatic conditions within the apparatus, thus avoiding heat leakage and the consequent necessity for corrections. The interior of the instrument, or calorimeter proper, is completely surrounded by jacketing walls through which water is circulated from a cold water tap and hot water boiler, so that the temperature can be regulated during the determination. By maintaining the jacket at a temperature corresponding with that of the calorimeter, all heat losses due to radiation are eliminated.

[1] MARTIN, E. V. Improved drying oven for plant material. Plant Physiol. 9: 1934.

FIG. 1. Adiabatic calorimeter with motor and water heater.

Within the jacket is a metal casing, oval in form, which is so fitted as to allow a minimum air space between it and the oval bucket which is placed within it. The bucket is nickel-plated and highly polished to decrease thermal exchanges by radiation. It holds the 2 liters of water, the stirrer, and the thermometer.

For the determination, the pellets are weighed in a weighing bottle and then placed in a capsule which is suspended in the bomb. Both the bomb and the capsule are made of illium alloy, which resists the action of the nitric and sulphuric acids formed as a result of combustion. One of the necessary conditions is the avoidance of secondary reactions involving the oxidation or solution of the material of which the bomb is made.[2]

The fuse wire is adjusted so that it makes contact with the terminals and pellets. Then the illium bomb is filled with oxygen to 25 lb. pressure, placed in the calorimeter bucket and covered with 2 liters of distilled water. The lid to the calorimeter is pushed forward and closed, and the motor started in order to stir the water surrounding the bomb and jacket. When the switch is closed that makes the electrical connection, the fuse ignites

[2] For specific directions and details see Booklet 106, The oxygen bomb calorimeter. Standard Calorimeter Co., East Moline, Illinois.

the pellets in the capsule and is itself burned. From the rise of temperature of the water in the bucket and the grams dry weight of plant material in the pellets used, the number of calories of energy involved is calculated.

It is necessary to make corrections for the heat produced by the fuse wire, which for the kind of wire employed is 2.4 calories per cm. Because of the use of pure oxygen at high temperature and pressure, certain reactions take place that do not occur in the ordinary process of combustion. Thus the free nitrogen in the small amount of residual air present upon closing the instrument is partially oxidized to N_2O_5, which combines with the moisture present in the bomb to become HNO_3. Similarly the nitrogen of the plant material oxidizes to a greater or less extent to HNO_3. The sulphur in the pellets, which under ordinary conditions of combustion is oxidized to SO_2, is converted in the calorimeter to SO_3, and this to H_2SO_4.

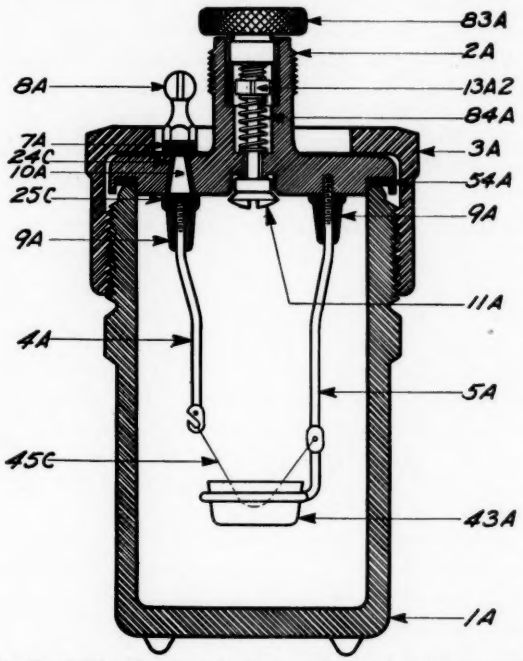

FIG. 2. Details of illium bomb: 1 A, illium cylinder; 2 A, illium cover, held in place by 3 A; 3 A, steel screw cap; 4 A, 5 A, electric terminals for fuse wire; 7 A, top terminal insulator; 8 A, top terminal; 9 A, illium locknut; 10 A, terminal cone; 11 A, illium valve; 13 A2, valve nut; 43 A, illium capsule for pellets; 54 A, cover gasket; 83 A, valve thumb nut; 84 A, valve spring; 24 C, cone insulator; 25 C, mica washer; 45 C, special fuse wire.

Since these acids represent a small portion of the energy produced, the bomb is rinsed out with distilled water, and the washings titrated with a standard solution of sodium carbonate made up of such a strength that each cc. represents one calorie. The calculations for a typical determination are shown in the example given here.

— Example —

$$\frac{\text{Water equivalent of calorimeter} \times \text{temp. rise °C.} - \text{corrections (wire + acid)}}{\text{Grams dry weight pellets}} = \text{calories per gram dry weight}$$

$$\frac{2450 \times 1.61 - 22.9}{1.1625} = 3365 \text{ calories per gram dry weight.}$$

The water equivalent of the calorimeter represents the amount of heat absorbed by the whole apparatus, including the 2 liters of water, per degree rise of temperature. It is found by combusting a standard substance of known heat value. The substances most commonly used with the values recognized by the United States Bureau of Standards are:

Benzoic acid	6320	calories per gm.
Naphthalene	9622	" " "
Cane sugar	3949	" " "

The water equivalent for the calorimeter used for the preceding example was found to be 2450. Unless otherwise stated, the small calorie is used here. It represents the amount of heat required to raise one cc. of water through 1° C. A large calorie is the equivalent of 1000 small calories.

The values in large calories for some of the organic compounds contained in plants were found to be as follows: Para rubber 10.2, resin 8.4, pure cellulose 4.0, and starch 3.8. Since there are many forms of resin, rubber, etc., these values must be considered as specific only for the plants from which they were taken, but they do represent the general range of values to be found in such materials. In consequence, when determinations show high caloric values it is usually an indication that the material contains an unusual amount of hydrocarbons, since each gram of these compounds has more than twice the value of cellulose, sugar, starch, and protein, the other principal components of plant tissues.

The number of grams that may be combusted at one time is determined not only by the size of the illium capsule, but also by the caloric value of the material. Three pellets weighing a total of 1 to 2 gm. make a convenient amount for each combustion. The thermometers used with this calorimeter read from 65° to 90° F., in 1/20 degree divisions. Two grams of *Helianthus annuus* seed when combusted in the calorimeter, give a rise of about

10° F., which would go beyond the limits of the thermometer if the initial temperature were above 80° F.; hence a safe rule is to limit the amount of material for each combustion to a maximum of 2 gm. and to have the initial temperature of the water within the thermometer range.

After combustion, the oxides of the mineral salts in the plant are found in the capsule as ash. In the giant cactus (*Carnegiea gigantea*), the ash of certain parts of the plant may be as high as 25.5 per cent. of the dry weight. Leaves of *Mesembryanthemum aequilaterale* contain about 25 per cent. of ash, while those of *Rosa, Rubus,* and *Prunus* yield approximately 6 per cent. ash on the same basis. In cases where the caloric value is not of special interest, but where it is desirable to know the amount of ash in various plant parts, the determinations may be made as indicated without recording the temperature rise. Ash analyses may also be made from the residue in the capsules when that is desirable. For example, table I gives WOLFF'S[3] analyses for the ash of *Helianthus annuus.*

TABLE I

COMPOSITION OF ASH OF *HELIANTHUS ANNUUS* IN PERCENTAGE

	K_2O	Na_2O	CaO	MgO	Fe_2O_3	P_2O_5	SO_2	SiO_2	Cl
	%	%	%	%	%	%	%	%	%
Seeds and fruits ...	16.23	7.41	7.63	12.29	1.6	35.43	2.34	14.65	2.42
Whole plant	60.77	1.84	12.56	6.75	0.22	8.89	1.71	0.88	6.38

Calorimetric determinations were made upon seeds of a number of species and life forms in order to ascertain the energy stored in each and to permit a comparison of the relative caloric values. The task of drying seeds before preparing the pellets is much simpler than with more succulent material, since respiration is at a minimum and the material is comparatively dry at the start. Combustion proceeds readily enough when whole seeds are used, but better average results were obtained from pellets of ground material. Table II gives the results obtained for the seeds of a number of crop plants and native conifers.

The great differences in growth during the early seedling stages, when the plant is dependent on the food stored in the seed, are more readily understood when one compares the energy supply upon which these plants may draw. For instance, *Pinus lambertiana* averaged 1222 calories per seed, 50 times as much as in *Pinus contorta murrayana.* The average for *Ricinus communis* was 2975 calories, or 2.4 times that of sugar pine and 20 times as much as for seeds of *Avena sativa.*

[3] WOLFF, EMIL. Aschen Analysen. 1871.

TABLE II

CALORIC AND ASH VALUES FOR VARIOUS SEEDS

SPECIES	CALORIES PER GM. DRY WEIGHT	CALORIES PER SEED	ASH
			%
Avena sativa	4238.3	143.4	2.5
Zea mays	4415.2	.....	.....
Sorghum vulgare	4017.6	144.6	1.2
Triticum sativum	4282.3	.....	0.8
Phaseolus coccineus	4282.2	.....	.....
Medicago sativa	5068.9	.....	.....
Cannabis sativa	5890.7	122.9	3.1
Brassica nigra	6049.5	.....	3.6
Helianthus annuus	6759.2	.....	.....
Ricinus communis	6834.7	2975.6	2.3
Pseudotsuga taxifolia	5998.3	59.9	1.5
Pinus ponderosa	5625.0	192.4	2.7
Pinus contorta murrayana	5989.2	26.7	2.7
Pinus lambertiana	6480.4	1221.8	1.7
Pinus flexilis	7117.7	372.9	2.6

The calorimetric method has been applied on an extensive scale to the plants obtained from the transplant and adaptation experiments carried on at the Alpine Laboratory and at Santa Barbara. Some representative results drawn from these are given in tables IV–IX. In addition, various series of determinations have been made for purposes of orientation, a typical example of which is furnished in table III, which records the caloric values for the largest individual of a widely spread group of cultivated sunflowers. At the time this plant was used, the achenes of the single large head were mature but not dry, while the lower leaves were slightly torn and beginning to turn brown. The leaves were cut in the order of position and labeled; they were weighed at once and the area taken by means of blue prints before drying. The stem, which was 2 meters tall, was cut into small pieces after weighing to insure rapid drying in the oven, and root and flower head were treated likewise. Each of these yielded much more material than needed for pellets, but an aliquot of each was utilized to secure a representative result (table III).

There proved to be less uniformity in the area, weight, and caloric value of adjacent leaves than was expected from their general appearance, a diversity probably due partly to shading and partly to competition between the leaves. Weights and calories rose more or less irregularly from a minimum at the bottom to a maximum in the upper third, to fall off rapidly to the top. By contrast, water gave the highest values in the lower third,

TABLE III

COMPARATIVE VALUES OF LEAVES AND ORGANS OF *HELIANTHUS ANNUUS*

LEAF	LEAF WEIGHT		WATER	ASH	LEAF AREA ONE SIDE	CALORIES PER GM. DRY WEIGHT	CALORIES IN WHOLE LEAF	RELATIVE VALUES
	WET	DRY						
	gm.	*gm.*	%	%	*sq. cm.*			
1 (top)	15.5	3.7	76.1	12.7	337	3993.2	14.780	43.8
2	19.6	4.3	78.0	12.4	339	3999.4	17.200	51.0
3	18.6	3.8	79.6	12.5	426	3971.0	15,100	44.8
4	38.8	8.2	78.9	11.4	770	3934.1	32,250	95.6
5	30.1	5.2	82.7	10.5	651	4051.6	21,100	62.5
6	36.1	7.5	79.3	11.8	709	4017.5	30,100	89.2
7	40.1	8.1	79.8	11.1	820	3907.9	31,600	93.6
8	24.7	..5	81.8	13.7	526	3932.7	17,700	52.5
9	42.8	8.6	80.0	12.0	798	3922.7	33,750	100.0
10	29.9	5.5	81.6	12.5	629	4040.6	22,230	65.9
11	25.9	5.2	80.0	12.1	509	3935.2	20,740	60.6
12	33.7	6.6	80.5	12.7	648	3936.3	25,970	76.9
13	20.9	3.8	81.7	11.7	494	3896.3	14,800	43.8
14	29.3	5.7	80.6	12.8	583	3881.2	22,130	65.5
15	27.2	5.4	80.2	13.4	536	3821.4	20,650	61.2
16	17.4	2.9	83.3	13.4	420	3912.1	11,350	33.6
17	17.5	3.2	81.8	14.7	416	3866.9	12,390	36.7
18	17.1	3.0	82.5	14.8	403	3826.7	11,490	34.0
19	17.1	2.3	86.5	14.9	393	3742.4	8,610	25.6
20	16.2	2.6	84.0	15.7	392	3662.3	9,530	28.2
21	19.3	4.4	77.2	14.3	440	3769.6	16,600	49.2
22	12.8	2.4	81.2	16.6	296	3720.2	8,350	24.9
23	14.3	2.9	79.6	16.6	350	3557.7	13,310	39.4
24	11.9	2.3	80.6	16.4	283	3520.7	8,100	24.0
25	11.3	2.1	81.5	17.4	274	3546.7	7,450	22.1
26	8.2	1.4	82.9	17.9	220	3424.8	4,795	14.2
27	9.1	1.7	81.3	18.7	234	3497.6	5,950	17.6
Average	22.4	4.35	80.9	13.9	480	3825.5	16,954	.....
Whole plant	3638.8	578.8	82.8	8.8	.....	3983.5	2,320,964	100.0
Leaves	605.4	117.3	80.9	13.9	12,956	3825.5	457,725	19.7
Stem	1342.0	204.4	84.5	7.3	195 cm. high	3850.0	786,940	33.9
Root	419.4	87.5	79.2	8.8	.....	3951.0	345,713	14.8
Head	1272.0	169.6	86.6	5.2	30 cm. diam.	4307.7	730,586	31.6

TABLE IV

RELATION OF TOTAL CALORIES TO INTENSITY OF COMPETITION

SPECIES	DRY WEIGHT PER PLANT	WATER	ASH	CALORIES PER GM. DRY WT.	TOTAL CALORIES PER PLANT	RELATIVE VALUES
	gm.	%	%			
Helianthus annuus (young)						
4 normal	11.8	89.2	15.3	3807	44,923	100.0
16 "	9.5	86.0	15.0	3701	35,160	78.2
64 "	9.8	83.6	15.4	3664	35,907	79.9
128 "	7.2	90.5	16.7	3600	25,920	57.7
Helianthus annuus (mature)						
4 normal	352.5	83.2	8.9	3835	1,351,838	100.0
16 "	119.0	83.2	7.5	3934	468,146	34.6
16 suppressed	54.6	83.5	7.6	3897	212,776	15.7
64 normal	59.8	84.1	10.2	3813	228,017	16.8
64 suppressed	1.2	91.1	9.4	3819	4,583	0.3
128 normal	45.7	84.5	8.5	3799	173,614	12.8
128 suppressed	16.1	79.0	9.6	3717	59,844	4.4
Triticum sativum (young)						
4 normal	4.8	83.1	14.3	3585	17,208	100.0
16 "	3.4	83.3	25.1	3491	11,869	68.9
64 "	2.3	81.6	25.5	3463	7,965	46.2
Triticum sativum (mature)						
4 normal	65.5	66.0	4.2	4042	264,751	100.0
16 "	50.5	63.0	3.5	4103	207,202	78.2
64 "	11.6	58.0	8.6	3828	44,405	16.7
128 "	8.4	54.0	14.5	3594	30,190	11.4
Zea mays (young)						
4 normal	42.3	80.0	10.8	4006	169,454	100.0
16 "	20.7	86.3	12.3	3868	80,068	47.2
64 "	24.3	84.3	10.9	3955	86,107	50.8
128 "	18.1	86.4	8.2	3747	67,821	40.0
Zea mays (mature)						
4 normal	241.7	84.9	3.5	4053	979,610	100.0
16 "	99.5	75.2	5.1	3961	394,120	40.0
16 suppressed	27.2	72.7	4.1	3899	106,053	10.8
64 normal	44.3	78.1	4.6	3909	173,169	17.6
64 suppressed	10.6	80.7	10.1	3828	40,577	4.1
Campanula medium						
4 normal	310.7	76.0	10.4	4498	1,397,529	100.0
64 "	37.0	78.0	10.6	3834	141,969	10.1
Plantago lanceolata						
4 normal	283.7	74.0	15.0	3628	1,029,264	100.0
64 suppressed	5.2	70.0	19.4	3507	18,236	1.7

and ash at the bottom, the latter decreasing rather regularly and the former rather irregularly to the top. The caloric value per gram was highest in the head, owing to the storage of oil in the seeds.

For the study of adaptation under different degrees of competition, a number of cultures have been established each year, with frequent changes of the species employed. Each culture contained four units 4 ft. square, separated by paths 1.5 ft. wide, in which the respective densities were 4, 16, 64, and 128 plants. The bed with four individuals served as a standard for the other three, in each of which the density was sufficient to produce a smaller or larger number of suppressed plants (table IV).

As would be expected, the young plants showed less response to crowding, since the demands of each individual had not reached the maximum. The mature plants yielded consistent and striking results throughout, the three crop species giving nearly identical relative values in the two higher densities. The suppressed plants were naturally more variable, but for the most part averaged about a fourth of the value for the normal or dominant individuals.

In all competition cultures without a buffer margin, the outermost plants profit from the lack of competition on the edge, the individuals at each corner often being especially well grown. Conditions as to water content and nutrients are much the same on all four sides, but the light intensity is distinctly higher on the south (table V).

The plants on the south margin were approximately 2.5 times higher in calories than were those on the north, primarily as a consequence of

TABLE V

TOTAL CALORIES PRODUCED UNDER COMPETITION AT SAME DENSITY

Helianthus annuus	Weight of plant		Water	Ash	Calories per gm. dry wt.	Calories per plant	Relative values
	Wet	Dry					
	gm.	*gm.*	%	%			
South margin	938.2	152.8	83.7	7.1	4071	622,049	100.0
North margin	416.1	61.5	84.9	8.1	3925	242,003	38.9
North suppressed	261.4	34.0	87.0	7.8	4018	136,612	21.9
Center dominant	121.1	21.3	82.4	7.8	3855	82,112	13.0
Center suppressed	83.1	9.8	85.8	8.0	3751	36,560	5.8

better illumination, while dominant plants on the north were about twice as large as the suppressed ones. The caloric values in the center were distinctly less for all individuals, while the suppressed were less than half as large as the dominants and contained but 5 per cent. as many calories as the plants on the south margin.

In seeking the functional explanation of the striking modifications and transformations secured under low light intensities, the calorimeter has proved an effective adjunct to measurements of transpiration and photosynthate. The species listed in table VI were grown in lath houses with light values of 12 to 4 per cent. of sunlight, the controls being located in sunshine.

The various species differ strikingly in their efficiency under the reduced intensities. The highest values in light of 12 per cent. were for *Solanum*, *Verbesina*, and *Mentzelia*, with relative performances of 15, 14, and 13 approximately, while the lowest were for *Allium*, *Madia*, and *Helianthus*, all at about 1 per cent. The best relative production in 4 per cent. light was that of *Clarkia* at 7.4, followed closely by *Solanum* at 6.4; the poorest performance again was that of *Allium*, *Madia*, and *Helianthus*, with half or less of 1 per cent. The average production in the deeper shade was 2.7, in the lighter shade 6.3, these values being in fair agreement with the respective light intensities. The number of calories may also vary greatly from year to year in correspondence with climatic conditions, especially sunshine and temperature, as is evidenced by the results obtained with *Helianthus* and *Plantago* in successive years.

In the endeavor to ascertain the effects of different lengths of day on species growing in the experimental garden, dark tents were employed to reduce the time of exposure to daylight from 13 hours in the long-day series to 9 in the mid-day and 5 in the short-day series. The plants were regularly best-grown in the longer exposure, and flowering occurred about two weeks earlier, leaves and flowers both being deeper colored. The mid-day series was intermediate in all respects, except that the stems were tallest. In the short-day tents the plants were shortest and palest, the flowers fewer, smaller and paler, blooming later than in either of the other series. In all cases the results were similar to those obtained in the lath houses, and this fact is reflected in the values shown in table VII, although the exposure to direct sunlight gave higher amounts in practically all cases.

The relative values differ much for the different species, the highest being given by *Verbesina* and the lowest by *Zinnia*, thus reflecting their abilities to grow and become modified in the shade. In general terms, the average production in the mid-day series with two-thirds as much sunshine was a little more than one-third of that in the long-day; and in the short-day with four hours less, it was again slightly more than one-third.

TABLE VI

RELATION OF TOTAL CALORIES TO LIGHT INTENSITY

SPECIES	LIGHT VALUES	WEIGHT OF PLANT		WATER	ASH	CALORIES PER GM. DRY WT.	CALORIES PER PLANT	RELATIVE VALUES
		WET	DRY					
	%	gm.	gm.	%	%			
Allium cepa	Sun	348.0	35.0	90.0	11.1	3777	132,195	100.0
	12	3.1	0.22	93.0	16.9	3762	817	1.0
	4	1.7	0.13	92.0	20.6	3552	462	0.6
Clarkia elegans	Sun	225.0	45.0	80.0	5.8	3980	179,100	100.0
	12	16.5	3.8	74.0	9.7	3821	14,520	8.7
	4	19.0	3.6	79.0	8.1	3757	13,525	7.4
Gilia capitata	Sun	317.5	81.5	74.0	6.0	4204	342,626	100.0
	12	12.1	2.3	81.0	7.4	4074	9,370	2.7
	4	4.2	0.9	79.0	14.1	3835	3,452	1.0
Helianthus annuus	Sun	950.6	217.7	77.1	5.7	3996	869,929	100.0
(1930)	12	25.0	2.1	91.6	10.4	3645	7,655	0.8
	4	5.5	0.16	97.1	10.4	3625	580	0.06
Helianthus annuus	Sun	602.0	149.2	75.0	4.5	3955	590,086	100.0
(1931)	12	52.3	6.1	88.0	12.8	3593	22,527	3.8
	4	8.7	1.2	86.0	16.6	3348	4,018	0.6
Madia elegans	Sun	1,260.0	394.1	69.0	8.5	4175	1,645,368	100.0
	12	24.9	5.3	79.0	18.6	3464	18,359	1.1
	4	11.0	2.3	79.0	20.5	3286	7,558	0.4
Mentzelia lindleyi	Sun	179.1	24.0	86.8	14.6	3581	88,344	100.0
	12	35.3	3.5	90.1	21.0	3323	11,631	13.1
	4	7.6	1.0	86.9	26.4	3110	3,110	3.5
Plantago lanceolata	Sun	149.3	22.4	85.0	14.3	3641	81,558	100.0
(1930)	12	11.1	1.4	87.4	13.7	3502	4,903	6.1
	4	10.7	0.9	92.0	19.0	3368	3,031	3.5
Plantago lanceolata	Sun	520.0	133.0	74.0	11.3	3826	508,858	100.0
(1931)	12	46.1	4.1	91.0	21.5	3202	13,128	2.5
	4	34.9	3.1	91.0	21.5	3127	9,694	1.8
Rumex patientia	Sun	279.3	58.8	79.0	11.3	3834	225,439	100.0
	12	16.1	3.9	76.0	18.3	3707	14,457	6.4
	4	7.2	1.0	86.0	19.4	3681	3,681	1.6
Solanum tuberosum	Sun	200.7	50.1	75.0	13.3	3819	191,332	100.0
	12	84.3	8.8	90.0	20.1	3417	30,070	15.6
	4	42.2	4.1	91.0	24.1	3169	12,993	6.7
Verbesina encelioides	Sun	540.0	125.7	77.0	10.3	3788	476,152	100.0
	12	95.7	19.3	80.0	15.3	3433	66,257	13.9
	4	48.8	6.7	86.0	21.2	3082	20,649	4.0

TABLE VII

RELATION OF TOTAL CALORIES TO LENGTH OF DAY

SPECIES	LIGHT	WEIGHT OF PLANT		WATER	ASH	CALORIES PER GM. DRY WT.	CALORIES PER PLANT	RELATIVE VALUES
		WET	DRY					
		gm.	*gm.*	%	%			
Gilia capitata	Long-day	317.5	81.5	74.0	6.0	4204	342,626	100.0
	Mid-day	181.5	36.7	80.0	11.1	3705	135,974	39.7
	Short-day	55.5	11.0	80.0	19.0	3482	38,302	11.1
Phacelia grandiflora	Long-day	223.0	58.0	74.0	18.5	3446	199,868	100.0
	Mid-day	60.5	13.3	78.0	20.4	3311	44,042	22.0
	Short-day	38.0	8.5	78.0	26.4	3094	26,297	13.1
Rumex patientia	Long-day	279.3	58.8	79.0	11.3	3834	225,439	100.0
	Mid-day	102.0	27.5	73.0	12.4	3774	103,785	46.0
	Short-day	21.8	3.8	83.0	15.6	3688	14,014	6.2
Verbesina encelioides	Long-day	117.4	25.3	78.5	11.5	3701	93,653	100.0
	Mid-day	142.1	20.7	85.4	11.5	3563	74,100	79.1
	Short-day	71.7	9.3	87.1	13.8	3488	32,439	34.6
Zinnia grandiflora	Long-day	88.0	18.8	79.0	11.3	3809	71,600	100.0
	Mid-day	9.8	2.4	75.0	13.9	3567	8,561	11.9
	Short-day	8.8	1.6	82.0	21.0	3247	3,247	4.5

TABLE VIII

RELATION OF TOTAL CALORIES TO AMOUNT OF NUTRIENTS

SPECIES	NUTRIENT RATIO	WEIGHT OF PLANT		WATER	ASH	CALORIES PER GM. DRY WT.	CALORIES PER PLANT	RELATIVE VALUES
		WET	DRY					
		gm.	*gm.*	%	%			
Calendula officinalis	2N : 2W	694.5	120.0	83.0	11.9	3871	464,520	100.0
	1N : 1W	166.5	37.5	78.0	16.2	3742	140,325	30.2
	0N : 0W	100.5	18.2	82.0	17.0	3608	65,666	14.1
Gilia capitata	2N : 2W	635.1	185.5	71.0	5.8	4268	791,714	100.0
	1N : 1W	102.5	28.7	72.0	5.1	4282	122,893	15.5
	0N : 0W	68.1	18.2	73.0	5.7	4205	76,531	9.5
Madia elegans	2N : 2W	253.5	103.0	59.0	14.7	3950	406,850	100.0
	1N : 1W	110.0	45.5	59.0	13.6	3801	172,946	42.5
	0N : 0W	68.5	21.6	68.0	18.9	3519	76,010	18.6
Petunia hybrida	2N : 2W	56.7	10.5	82.0	14.4	3747	39,344	100.0
	1N : 1W	55.5	9.8	82.0	18.8	3548	34,770	90.7
	0N : 0W	8.8	1.7	80.0	24.8	3564	6,059	15.8
Solanum tuberosum	2N : 2W	235.0	58.5	75.0	9.5	3932	230,022	100.0
	1N : 1W	121.0	26.9	78.0	12.5	3805	102,355	44.4
	0N : 0W	42.0	8.8	79.0	16.2	3783	33,290	14.4

In the series designed to test the response to increased nutrients, commercial fertilizer was applied to one row of beds in the amount regularly used by gardeners for the soil concerned, and in twice the amount. The supply of water was similarly increased to maintain the soil solution at about normal density, while in the control row no addition was made to the amount received from rainfall, and no nutrient was added (table VIII).

As was to be expected, the addition of nutrients and water greatly increased the size of the plants in comparison with the controls, the number of shoots being greater and the leaves larger and more deeply colored. The difference between the several species was marked, the maximum effect being found in *Gilia* with 11 times the control and the minimum in *Madia* with 5.5 times.

The soil series consisted of soil pits in three rows, one containing fertile garden loam, another a fertile black clay called adobe, and a third, sand. While soil is the remote factor, the immediate response is to water content, the fluctuations of which are automatically regulated by soil texture under the condition of equal watering (table IX).

TABLE IX

RELATION OF TOTAL CALORIES TO TYPE OF SOIL

SPECIES	SOIL	WEIGHT OF PLANT		WATER	ASH	CALORIES PER GM. DRY WT.	CALORIES PER PLANT
		WET	DRY				
		gm.	*gm.*	%	%		
Madia elegans	Loam	1,260.0	394.1	69.0	8.5	4175.0	1,645,368
	Adobe	422.0	182.5	57.0	8.1	4105.0	749,163
	Sand	275.5	111.8	60.0	14.9	3973.0	433,000
Plantago lanceolata	Loam	480.0	106.4	78.0	8.3	4008.2	426,472
	Adobe	334.5	70.7	79.0	13.5	3709.4	263,254
	Sand	65.0	9.6	85.0	13.6	3319.8	31,870
Solanum tuberosum	Loam	200.0	50.1	75.0	13.3	3819.4	191,352
	Adobe	30.0	7.5	75.0	6.8	3809.5	28,580
	Sand	15.8	1.3	80.0	7.4	3795.6	4,930

In all three species selected, the growth in loam was two to several times that in the adobe, and growth in the latter two to eight times that in the sand. In general the soil series yielded the most consistent and striking modifications in response to varying water content.

From the preceding data it is evident that the calorimetric method introduces into ecology and the related sciences, agriculture, horticulture, forestry, etc., an accurate chemical procedure for measuring performance. It makes it possible to ascertain in exact terms not only how much energy is utilized, but also how much is stored in seed, bud, or underground part

against the time of future need. The efficiency of the individual plant, variety, or species may be compared with that of others grown under the same conditions, and thus serve as a definite basis for selection or breeding. The plant itself may be employed as an index of the energy available at different altitudes or latitude, under various natural canopies or artificial covers, or in connection with long-day and short-day exposures, as in the work of GARNER and ALLARD. It also becomes possible to obtain the plant's reaction to the influence of varying amounts of water, in the case of irrigated crops for example, of soil nutrients and fertilizers, of the air content in the case of water-logged soils, and so forth. The effect of the time and intensity of grazing range grasses or time of cutting fields of alfalfa can be measured with signal accuracy, and perhaps even a forecast made of the next season's growth on the basis of the amount of stored food.

Furthermore, two processes of fundamental importance in plant life, competition in the community and adaptation in the individual, can be evaluated more accurately and objectively by means of the calorimeter. The degree of competition between plants determines not only the amount of energy available for each, but also the conditions under which it can be utilized, while the energy actually transformed represents the performance of the several competitors.

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CARBON DIOXIDE CONTENT OF THE GAS FROM PEA PODS

Z. I. KERTESZ

(WITH ONE FIGURE)

Introduction

Shelled green peas deteriorate very rapidly. Respiration (**10**) and enzymes (**7, 8**) are mostly responsible for these objectionable changes, which cause a material loss in many canneries. Several studies on the prevention of this deterioration have been reported (**8, 9, 14**); but in spite of the well known fact that peas stored in the pods (*i.e.*, in a natural gas mixture) keep better than shelled peas, no method has been proposed for the storage of shelled green peas in a gas.

Gas storage of fruits and vegetables has been studied by several investigators (**9, 15**). Experiments with the storage of shelled green peas in a gas similar to that contained by the pods may offer a possibility for the prevention of deterioration of the peas between shelling and canning. The writer presents the results of investigations on the composition of the gas contained in the pods of green cannery peas (*Pisum sativum* L. variety Perfection).

Earlier workers (**11,** p. 391) were of the opinion that the systematic arrangement of plant organs precludes a deficiency of oxygen in plants, and held that carbon dioxide is absent in them (**2,** III, p. 19). Other investigators proved this assumption faulty, however, and found carbon dioxide in the different organs of plants. LUMIA (**2,** III, p. 19) showed that gas samples obtained from unripe figs contained 5.25 per cent. carbon dioxide and only 17.92 per cent. oxygen. NEGRI (**2,** III, p. 19) found 9.88 per cent. carbon dioxide and 16.59 per cent. oxygen in the gas from immature fruits of *Gomphocarpus,* while the gas from the ripe fruits contained 3.48 per cent. carbon dioxide and 23.15 per cent. oxygen. DEVAUX (2, III, p. 20) analyzed the air from the hollow inside of a pumpkin (*Cucurbita maxima*) and found 2.52 per cent. carbon dioxide, 18.29 per cent. oxygen, and 79.19 per cent. nitrogen. MALAQUIN (**2,** III, p. 19) found in the gas from the pods of *Colutea,* 6.9 per cent. carbon dioxide and 14.3 per cent. oxygen. MAGNESS (**13**) observed that the gas in the intercellular spaces of apples contains the following percentages of carbon dioxide: About 7 per cent. when stored at 6° C., 12 per cent. at 11° C., 17 per cent. at 20° C., and 21 per cent. at 30° C. At the same time there was a corresponding decrease in the oxygen content of the samples. He found the same general relation to exist in potatoes and carrots. DAVIS (**5**) found in the gas samples from potatoes a carbon dioxide content of 5–6 per cent. and 10–11 per

cent. of oxygen when they were stored at 17°–18° C. With increasing storage temperature the carbon dioxide content and the respiratory ratio enlarged rapidly. Recently DANIN (**4**) studied the gas contained in the cenobia of *Rivularia polyotis*, and found that light intensity influenced the oxygen content, which reached 70 per cent. under certain circumstances. COLLA (**3**) investigated the carbon dioxide and oxygen contents of different organs of *Nymphaea alba*, and found an increased oxygen content in the roots and stems as a result of illumination. During the night a decrease in oxygen content of the gas in the leaves was observed, while only insignificant changes in the carbon dioxide content occurred. In the roots, however, he found a decrease in the carbon dioxide content during the day and an increase at night.

Besides the normal constituents of air, such as oxygen, nitrogen, and carbon dioxide, the presence of other gases in plant organs has been reported also. CLOËZ and GRATIOLET, BOUSSINGAULT, and CORENWINDER (**2**, I, p. 522) were unable to find carbon monoxide in the plants studied by them, while LANGDON and GAILEY (**12**) showed the presence of carbon monoxide in the gas obtained from the pneumatocyst of the Pacific coast kelp, *Nereocystis leutkeana*. The presence of hydrocarbons (methane ?) in plants has also been reported (**2**, I, p. 522).

It is evident, therefore, that carbon dioxide is often present in the gas contained in plants or in plant cavities. No information could be obtained from the literature on the composition of the gas contained in pea pods.

Experimentation

PRELIMINARY WORK AND METHODS USED

The carbon dioxide content of the gas contained in the pods of samples of commercial peas shipped from California to Geneva, New York, was first determined. The samples were kept under constant refrigeration during shipment and were removed from the containers immediately upon arrival. The temperature of the peas was between 5° and 10° C.

The method used in the determinations was as follows: A separatory funnel of 100 cc. capacity was filled with water and placed with the tip upward in a large beaker filled with water. A common glass funnel was placed in the opening of the separatory funnel and under this the peas were shelled. The gas from the opened pea pods bubbled up through the funnel into the separatory funnel. After all the pods of a sample were opened and the gas collected, the funnel into the opening of the separatory funnel was removed and replaced by a beaker of about 200 cc. capacity. This beaker was placed under the separatory funnel while the latter was still submerged in the water in the large beaker. The separatory funnel and the small beaker were then lifted out of the large beaker and carried to the

gas analysis apparatus. The whole operation was usually completed in three minutes.

The gas sample was sucked over into a Hempel precision gas burette, using potassium hydroxide to absorb the carbon dioxide and alkaline pyrogallol solution to absorb the oxygen present in the gas samples. All determinations were made at 26°–29° C., and no correction was made for temperature, which would affect only the values of "gas per pod." The results of the preliminary determinations are presented in table I.

TABLE I

CARBON DIOXIDE AND OXYGEN CONTENTS OF PEA PODS SHIPPED FROM CALIFORNIA TO GENEVA, N. Y. (CALIFORNIA TELEPHONE PEAS)

SAMPLE NO.	DATE	CO_2	O_2	O_2 CALCULATED	DIFFERENCE IN O_2	RESPIRATORY RATIO
		%	%	*cc.*	*cc.*	
1	April 23	0.91	20.40	21.00	0.60	1.52
2	April 28	0.52	19.96	21.00	1.04	0.48
3	May 13	0.72	19.78	21.00	1.22	0.56

As expected, the gas obtained from the pea pods contained carbon dioxide, the percentage varying from 0.52 to 0.91. Since the method for collecting the gas samples gave satisfactory duplicate results in several trials, it was used for all determinations reported here.

In table I, as also in several following tables, values are presented for the respiratory quotient. The calculation of these values is based on the assumption that pea pods contain normal air, and that the rate of diffusion for the different gases through the pods is the same. As far as the writer is aware, no data on the diffusion of different gases through pea pods have been published.

In the calculation of the quotients it was assumed that the air contained in the pods was of normal composition, containing 21 per cent. of oxygen by volume and 79 per cent. of other constituents (mostly nitrogen), and that the latter was not absorbed by the analytical solutions. From the residual amount of this fraction from the gas samples was calculated the amount of oxygen associated with it originally. This amount of oxygen is referred to as "O_2 calculated." The difference between this calculated amount and the amount of oxygen actually determined is referred to in the tables as "difference in oxygen." It might be said that this difference in oxygen represents the efficiency in oxygen determined in the pods at a certain time and not the amount of oxygen consumed during a certain period.

The ratio between the amount of carbon dioxide found and the difference in oxygen is designated in the tables as "respiratory ratio," although it must be realized that it represents the findings *at* a certain time and not the ratio of oxygen consumed and carbon dioxide produced *during* a certain period.

This ratio showed a wide variation in the samples used for preliminary determinations, but on account of the unknown origin and history of these samples no conclusions are drawn from the figures.

Changes in composition of gas of pea pods associated with growth

To obtain an insight into the changes that might be related to the growth and ripening of peas in the pods, several samples were collected at 8 A.M. at different dates during the 1932 season, from the same field of Perfection peas in Geneva, N. Y. These peas were sown on April 10 and maximum blooming occurred on June 20. On each harvest date large numbers of pods were picked and representative samples of 35 to 71 pods were chosen for analysis. The results of the determinations are presented in table II.

The average weight of pods increased during the early growth stages and then slowly decreased. This is in accordance with the findings of Bisson and Jones (**6**) on Dwarf Telephone peas. The amount of gas obtained per pod remained nearly constant in spite of the considerable changes in the size of the pods and of the peas in the pods. The percentage of carbon dioxide in the gas samples obtained was also fairly constant.

The changes in the sugar content of growing peas have been studied by several investigators (**1, 6, 8**). All have found that the maximum sugar content is attained at a relatively early stage of ripening, much before the peas are considered ripe for canning. The decrease in sugar content continues until the peas are dry. The rate of respiration decreases in growing peas presumably as a result of the diminishing sugar content. On the basis of these facts, therefore, it was reasonable to suppose that the carbon dioxide content in the pea pods would decrease during ripening, but the results presented in table II show no such essential change. This may be explained by assuming a changing rate of diffusion of the gases through the pods, depending upon the relative concentration of carbon dioxide inside. While the rate of respiration undoubtedly changes during growth, the rate of absolute or specific diffusion seems to be regulated by some factors in such a manner that a roughly constant carbon dioxide content is maintained in the pods. The respiratory quotient varied from 0.34 to 0.57.

Diurnal changes in carbon dioxide content of pea pods

The fact that no change in the carbon dioxide content of the gas in pea pods during growth could be established raised the question as to whether

TABLE II

CARBON DIOXIDE AND OXYGEN CONTENTS OF PEA PODS AS INFLUENCED BY CHANGES IN GROWTH; ALL SAMPLES HARVESTED AT 8 A. M.

SAMPLE NO.	DATE (JULY)	SIZE OF PODS	AVERAGE LENGTH	NO. OF PODS	AVERAGE WEIGHT OF PODS	TOTAL GAS OBTAINED	TOTAL GAS PER POD	CO_2	O_2	CALCULATED O_2	DIFFERENCE IN O_2	RESPIRATORY RATIO
			mm.		*gm.*	*cc.*	*cc.*	%	%	*cc.*	*cc.*	
1	3	Medium	59	45	3.54	45.30	1.01	1.55	18.56	9.63	1.23	0.57
2	5	Medium	.....	71	.....	67.00	0.95	1.72	18.14	14.28	2.13	0.54
3	5	Large	68*	53	5.76	46.70	0.88	1.61	16.93	10.11	2.21	0.34
4	6	Large	.....	53	5.49	61.20	1.15	1.88	17.90	13.05	2.10	0.55
5	13	Large	.....	35	4.47	39.55	1.13	1.14	.....	.....	.....	.....
		Average	.....	.....	.....	.....	1.02	1.58	17.88	.....	.....	0.50

* Cannery ripe.

the carbon dioxide content varied over a 24-hour period. One set of determinations, presented in table III and in figure 1, proved that marked changes in the carbon dioxide content do occur during a 24-hour day.

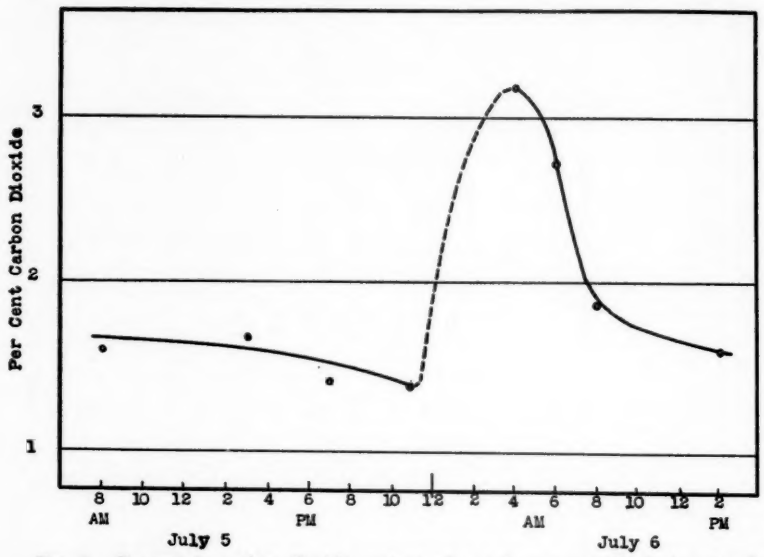

FIG. 1.—Changes in carbon dioxide content of gas from pea pods during the day and at night.

For these experiments Perfection pea pods were used from the same field from which all the material for the other experiments (except the preliminary ones) was collected. The average weight of a single pod varied from 5.19 to 5.95 grams, giving a mean weight of 5.62 grams. The "gas per pod" was about the same as in the previous experiment, ranging around 1 cc.

The percentage of carbon dioxide in the gas sample taken from pods at 8:00 A. M. on July 5 was 1.61. This value decreased somewhat later in the day, reading at 11:00 P. M. 1.39 per cent. Unfortunately no determinations were made between 11:00 P. M. and 4:00 A. M. next day, when the percentage of carbon dioxide was 3.19. On July 6 the values for the carbon dioxide content of the gas decreased rapidly, and by 8:00 A. M. had reached almost the average in table II. During the day it decreased again, as it did on the previous day. The same relation between the time of day and the carbon dioxide content was found by COLLA (3) in the roots of *Nymphaea alba*. It is interesting to note that the

TABLE III

CHANGES IN CARBON DIOXIDE AND OXYGEN CONTENTS OF PEA PODS DURING THE DAY AND AT NIGHT

SAMPLE NO.	DATE (1932)	WHEN TAKEN	NO. OF PODS	AVERAGE WT. OF PODS	GAS OBTAINED	GAS PER POD	CO_2	CO_2	O_2	O_2	CALC. O_2	DIFF. O_2	RESPIRATORY RATIO
				gm.	*cc.*	*cc.*	*cc.*	%	*cc.*	%	*cc.*	*cc.*	
1	July 5	8 A. M.	53	5.76	46.70	0.88	0.75	1.61	7.90	16.93	10.11	2.21	0.34
2		3 P. M.	50	5.84	48.00	0.96	0.80	1.67	8.80	18.35	10.21	1.41	0.57
3		7 P. M.	60	.....	60.20	1.00	0.85	1.41	.....	.....	.....	.....	.....
4		11 P. M.	45	5.26	36.00	0.80	0.50	1.39	6.20	17.22	7.79	1.59	0.31
5	July 6	4 A. M.	53	5.71	50.20	0.95	1.60	3.19	.....	.....	.....	.....	.....
6		6 A. M.	53	5.95	49.40	0.93	1.35	2.73	6.45	13.06	11.06	4.61	0.29
7		8 A. M.	53	5.64	61.20	1.15	1.15	1.88	10.95	17.90	13.05	2.10	0.55
8		2 P. M.	53	5.19	61.70	1.16	1.00	1.62	11.25	18.25	13.15	1.90	0.53
		Average	.....	5.62	.....	0.98	.....	.....	.....	.....	.....	.....	.....

respiratory quotient did not vary in spite of the broad fluctuation of the carbon dioxide content in the gas samples.

The marked changes in the carbon dioxide content of the pods are undoubtedly due to several factors. It is reasonable to suppose that during the night, when translocation of the sugars takes place, the sugar content of the peas increases. This higher sugar content causes an increase in the rate of respiration (**11**), which results in higher percentage of carbon dioxide in the pods. The lack of photosynthesis during the night may be partly responsible for the higher carbon dioxide content found in the pods. Of course, the absolute or specific permeability of the pods may also change because of the changing temperature and moisture content of the surrounding air. This last factor, however, seems of minor importance in view of the immaterial changes observed during growth, although samples were often collected under widely different meteorological conditions.

Influence of short storage and freezing on carbon dioxide content of pea pods

For this experiment a great number of pods were picked on July 13, 1932, and divided into several groups containing 35 pods each. One group was opened as previously described and the gas analyzed at once, while duplicate samples were stored at + 25°, + 5°, – 5°, and – 20° C. for 18 hours. After this, one sample from each temperature was analyzed, while the other sample was stored at + 25° C. for an additional 6-hour period and then analyzed. This experiment was carried out twice with practically the same results. The results of the second experiment are presented in table IV.

The "gas per pod" always decreased during storage. The carbon dioxide content of the gas samples from the pods stored at room temperature increased up to 5 and 18 hours, but was decreasing again at 24 hours. In the samples stored at lower temperatures for 18 hours the carbon dioxide content was higher than in the samples kept at room temperature.

The frozen samples stored for an additional 6-hour period at room temperature showed further substantial increases in carbon dioxide content. This gain was more pronounced in the sample stored at – 20° than in that kept at – 5° for the 18-hour period. That the carbon dioxide in these samples increased after defrosting must be the result of the freezing, which partially destroys the structure of the peas and increases their rate of respiration.

It was of interest to note what part the permeability of the pods to gases plays in the composition of the gas held inside. For this purpose several samples were sealed with low-melting-point paraffin and stored similarly to those samples reported in table IV. As one would expect, during freezing the paraffin broke away from the frozen pods or was cracked to such an

TABLE IV

INFLUENCE OF STORAGE AND FREEZING ON CARBON DIOXIDE CONTENT OF PEA PODS

SAMPLE NO.	STORAGE CONDITIONS	AVERAGE WEIGHT OF PODS	GAS OBTAINED	GAS PER POD	CO_2
		gm.	*cc.*	*cc.*	%
1	No storage	4.47	39.55	1.13	1.14
2	5 hr. at +25° C.	4.71	30.35	0.87	1.81
3	18 hr. at +25° C.	4.97	27.20	0.78	2.20
4	24 hr. at +25° C.	4.86	26.00	0.74	1.89
5	18 hr. at +5° C.	4.60	31.40	0.90	2.71
6	Same, concluded by 6 hr. at +25° C.	4.82	29.65	0.85	2.19
7	18 hr. at −5° C.	4.59	24.70	0.71	4.46
8	Same, concluded by 6 hr. at +25° C.	4.67	22.45	0.64	10.90
9	18 hr. at −20° C.	4.55	32.05	0.92	4.53
10	Same, concluded by 6 hr. at +25° C.	4.49	30.65	0.88	17.93

extent that perfect closure could not be expected. This was especially true in samples 7 and 8 of table V. To overcome this difficulty one sample (no. 10) was frozen for 18 hours, and sealed only after defrosting at room temperature.

In all samples in which the pods were kept at room temperature for any length of time, the carbon dioxide content was 42.6 to 49.8 per cent. This concentration seems to be the limit for carbon dioxide production, although small amounts of oxygen were still present in the pods. The changed respiratory quotient indicates a disturbed respiration. Higher ratios were observed by several workers in the case of injured peas or unsuitable conditions (**5, 10, 11**).

Summary

1. During the growth of Perfection peas, no significant changes in the carbon dioxide content of the gas in the pods could be found when all of the samples were collected at 8 A.M. The average value was 1.6 per cent.

2. During the night the carbon dioxide content of the pea pods was doubled in the samples studied. The carbon dioxide content of the pods began to decrease as early as 6 A.M., and about 8 A.M. reached a level that was approximately maintained during the rest of the day. It is suggested that this higher carbon dioxide content of the pods during the night is due to the changed ratio of respiration to photosynthesis.

TABLE V

INFLUENCE OF PARAFFIN SEALING ON CARBON DIOXIDE CONTENT OF STORED AND FROZEN PEA PODS

SAMPLE NO.	STORAGE CONDITIONS	GAS OBTAINED	GAS PER POD	CO_2	CO_2	O_2	O_2	CALC. O_2	DIFF. IN O_2	RESPIRATORY RATIO
		cc.	*cc.*	*cc.*	%	*cc.*	%	*cc.*	*cc.*	
1	5 hr. at 25° C.	24.60	0.70	10.60	43.10	0.70	2.85	3.54	2.84	3.73
2	18 hr. at 25° C.	18.80	0.54	8.30	44.20	.....	.....	.....	.....	.....
3	24 hr. at 25° C.	22.20	0.63	10.00	45.40	.....	.....	.....	.....	.....
4	18 hr. at + 5° C.	23.30	0.67	3.90	16.73	.....	.....	.....	.....	.....
5	Same, concluded by 6 hr. at + 25° C.	22.30	0.64	9.50	42.60	0.95	4.26	3.15	2.20	4.32
6	18 hr. at − 5° C.	29.90	0.85	1.90	6.35	.....	.....	.....	.....	.....
7	Same, concluded by 6 hr. at 25° C. (sealed before freezing)	22.45	0.64	2.45	10.91*	.....	.....	.....	.....	.....
8	18 hr. at − 20° C., concluded by 6 hr. at 25° C. (sealed before freezing)	26.40	0.75	2.85	10.79*	.....	.....	.....	.....	.....
9	Same, sealed after cold storage, before 6 hr. period	40.00	1.14	19.90	49.75	0.70	1.75	5.16	4.46	4.46

* Samples imperfectly sealed because during freezing some of the paraffin came off.

3. When pods were frozen, the carbon dioxide content of the gas contained in them increased to 4.5 per cent., presumably on account of the higher respiratory activity caused by freezing. Changed permeability of the pods may also take part in this phenomenon. When the frozen peas were then stored at 25° C., this carbon dioxide increased to a value of 17.9 per cent.

4. When pea pods were sealed with paraffin and stored at room temperature or frozen and kept at room temperature afterward, the carbon dioxide content of the gas from the pods reached 43 to 50 per cent. This indicates that there must be a constant and very vigorous exchange of gases through the normal pods, in order to keep the carbon dioxide content as low as an average of 1.6 per cent.

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METABOLISM OF ETIOLATED SEEDLINGS AS AFFECTED BY AMMONIUM NUTRITION

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Introduction

Changes in the nitrogen relations of plants caused by altering the nitrogen supply have been studied by numerous investigators. These changes, however, must be correlated with the metabolism of other substances, especially carbohydrates, in order to understand better the nitrogen relationships.

The nitrogenous metabolism of the seedling concerns itself mainly with the decomposition of already existing proteins stored in the kernel, followed by the translocation and regeneration of the simpler substances into proteins at the growing points. A study of etiolated seedlings may be expected to yield information as to the intermediate products of protein metabolism, for protein regeneration is inhibited in the absence of light, owing primarily to insufficient carbohydrate supply.

PRIANISCHNIKOW and coworkers (**7, 8, 9**) have emphasized the importance of available carbohydrates in the nitrogen metabolism of etiolated seedlings supplied with ammonium salts; however, no carbohydrate determinations were made. The seedlings, grown in water cultures for a period of ten days in the dark, were classified into three groups:

I. Seedlings of the grass type (barley, maize) and the pumpkin when supplied with ammonium salts showed increases in total nitrogen and amides, but no increase in ammonia.

II. Seedlings of the starchy legumes (pea, vetch) increased in total nitrogen and amides only when ammonium salts were accompanied by calcium carbonate.

III. Seedlings of yellow lupine showed that nutrition with ammonium salts caused serious disturbances in the synthetic reactions, manifested by accumulation of ammonia and decrease of asparagine. Addition of calcium carbonate failed to restore the normal course of nitrogen metabolism.

The seeds used differ greatly with respect to the relative amounts of nitrogen free and nitrogen containing reserves (**7**). The respective ratios used as an index to these differences are as follows: grass type 6 : 1, starchy legumes 2 : 1, and yellow lupine 0.6 : 1. Artificial types were employed (**7, 14**) from which it was shown that utilization of ammonium salts was not a specific character of the plant, but depended upon the nutritive condition.

One of the chief criticisms concerning PRIANISCHNIKOW'S investigations is the method which he employed in preparing the plant material for analy-

sis. The seedlings were dried at 70° C., thus rendering his ammonia determinations misleading. Furthermore, he did not separate the principal storage organs from the remainder of the seedlings, thereby having no basis for determining the extent of protein decomposition in the seed and regeneration in the growing seedling. HUNTER (**3**), employing improved methods of chemical analysis, including carbohydrate determinations, carried out experiments similar to those by PRIANISCHNIKOW, and found that the response of *Cucurbita* seedlings to ammonium salts was very different from that reported by PRIANISCHNIKOW (**7, 8**), mainly with respect to the accumulation of ammonia; however, the response of *Phaseolus* was similar to that reported (**7, 8**) for the starchy legumes.

It is unfortunate that PRIANISCHNIKOW did not grow his seedlings in complete nutrient solutions, as the distilled water and the solution of ammonium salts used unquestionably had effects which would not have appeared had the usual ions been present (**15**). There is evidence (**10, 15**) that calcium must play a more prominent rôle in altering the response of seedlings to ammonium salts than was realized by PRIANISCHNIKOW when he planned his experiments.

Employing improved methods, this investigation involves a further study of the effects of ammonium nutrition on the carbohydrate and nitrogen metabolism of etiolated seedlings as modified by the type and amount of food reserves in the seed. The present paper reports an attempt to throw additional light upon certain fundamental questions: How and to what extent do seedlings utilize ammonium nitrogen? What determines the rate of ammonium absorption? What conditions are associated with ammonium injury?

Materials and methods

Seeds of the following species were chosen: *Cucurbita pepo, Phaseolus vulgaris, Lupinus luteus,* and *L. albus.* The first three species are representative of PRIANISCHNIKOW's three types respectively, while *L. albus* was selected as a possible intermediate between the second and third types. The composition of seeds of the various species as determined by SCHULZE (**12**) is partly given as follows (on the basis of dry weight of kernels): *Cucurbita* 33.07 per cent. protein, 55.20 per cent. ether extract; *Phaseolus* 24.06 per cent. protein, 43.98 per cent. starch; and *Lupinus luteus* 54.38 per cent. protein. SCHULZE and CASTORO (**13**) report *Lupinus albus* 43.06 per cent. protein.

After the usual precautions in the selection and germination of the seeds, young seedlings (3–4 cm. in length) were transferred to the culture solutions consisting of 0.006 M. $(NH_4)_2SO_4$, 0.0045 M. $MgSO_4 \cdot 7H_2O$, 0.0045 M. KH_2PO_4, and 0.0045 M. $CaCl_2$. A pH of 6.4 was obtained by addition of NaOH. The controls were made up similarly except that $(NH_4)_2SO_4$ was

omitted. The pH of the culture solutions was kept fairly constant by renewal at 48-hour intervals. The seedlings were grown in a dark room at 23°–25° C. In the first of the two series of experiments, the seedlings of the various species (except *Lupinus albus*) were grown in nutrient solutions for a period of ten days. In a second series the seedlings were grown until the ammonium seedlings showed injury.

The plant material was preserved and extracted with 80 per cent. alcohol. Nitrogen fractions were determined according to PHILLIPS *et al.* (**5**). After removing the alcohol from a portion of the alcoholic extracts, it was cleared and deleaded (**6**) before determining sugars. The bicarbonate modification of the Tompsett method as described by PHILLIPS (**4**) was employed in all carbohydrate determinations. Insoluble acid hydrolyzable polysaccharides and nitrogen were determined in the residue. Ether extract (**1**) was determined in the pumpkin seedlings.

Results

The results were calculated and studied on the basis of weight of constituents per hundred seedlings and are presented in part in table I. The results as expressed on other bases (**2**), not included here, may be calculated from data in table I. Conditions existing in the various species are more comparable when constituents are expressed as percentage fresh weight. Some correlations concerning ammonium absorption and utilization are presented in table II.

Discussion

Cucurbita pepo (*10 days*).—The increase in total nitrogen caused by ammonium was contributed largely by the ammonia fraction. The increase in amides was apparently due to a favorable carbohydrate-nitrogen balance; however, the increase was much less than that reported by PRIANISCHNIKOW (**7, 8**). Protein formation was favored by ammonium nutrition, but not at the expense of the protein reserves. Accumulation of reducing sugars in the stems and roots was remarkably checked by ammonium. Sucrose relations appeared to be somewhat obscure in this and other species studied.

The utilization of ether extract (oily reserve) by seedlings was somewhat retarded by ammonium nutrition, as shown by the following data (in percentage dry weight): Ten days: NH_4 cotyledons 5.40 per cent., control 4.88 per cent.; nineteen days: NH_4 cotyledons 4.49 per cent., control 3.67 per cent. The tendency to hinder utilization of the oily reserve was more pronounced in the 19-day ammonium injured seedlings. This injury was characterized by withering of the young leaves, which was also shown by ammonium injured *Phaseolus*. In all the species studied root discoloration was not associated with ammonium injury.

TABLE I

RESULTS OF CHEMICAL ANALYSES EXPRESSED AS WEIGHT PER HUNDRED SEEDLINGS

	Cucurbita 10 days		*Phaseolus* 10 days		*Phaseolus* 22 days		*Lupinus albus* 17 days		*Lupinus luteus* 9 days	
	NH_4	Control	NH_4	Control	NH_4	Control	NH_4	Control	NH_4	Control
Fresh weight	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Stems and roots	179.6	174.7	363.6	369.6	279.1	319.2	270.4	348.2	94.5	104.8
Cotyledons	44.1	40.3	14.0	14.6	3.1	3.0	81.0	80.5	56.8	57.9
Dry weight										
Stems and roots	5.80	5.96	22.33	22.16	16.92	18.77	13.22	14.02	4.59	5.06
Cotyledons	2.77	2.82	3.27	3.31	2.57	2.54	6.26	5.82	4.58	4.48
Total nitrogen	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Stems and roots	396.5	273.6	1709.1	1573.0	1690.0	1480.8	1612.3	1469.2	473.8	454.0
Cotyledons	194.1	174.1	77.2	81.5	73.5	60.9	626.5	561.8	546.9	538.4
Insoluble N										
Stems and roots	128.6	118.5	750.4	692.1	485.6	467.2	1128.2	1081.0	285.5	314.7
Cotyledons	139.4	122.9	59.2	60.5	53.0	43.5	443.0	392.9	311.5	306.2
Soluble N										
Stems and roots	267.9	155.1	958.7	880.9	1204.4	1013.6	484.1	388.2	188.3	139.3
Cotyledons	54.7	51.2	18.0	21.0	20.5	17.4	183.5	168.9	235.4	232.2
Amino N										
Stems and roots	132.0	112.4	423.8	401.7	361.3	325.2	205.6	190.8	91.0	86.6
Cotyledons	24.4	21.2	6.9	7.6	7.0	6.0	80.7	78.9	105.5	109.5
Amide N										
Stems and roots	21.1	15.8	173.8	166.3	171.6	171.2	81.0	45.4	34.3	37.4
Cotyledons	4.0	3.1	3.8	1.7	1.6	1.3	29.2	38.8	40.7	33.0
Ammonia N										
Stems and roots	84.6	14.1	33.0	18.0	133.0	37.2	102.0	14.8	35.7	6.2
Cotyledons	11.0	5.7	1.3	0.9	4.7	2.5	4.4	3.9	5.7	5.6
Reducing sugars										
Stems aud roots	56.6	138.8	1290.4	1208.0	24.5	36.7	65.6	72.1	22.9	27.5
Cotyledons	63.8	34.7	41.1	48.1	18.7	19.6	31.7	29.0	28.9	30.4
Sucrose										
Stems and roots	54.1	35.2	225.6	262.4	4.4	18.0	25.3	30.4	21.4	19.0
Cotyledons	13.0	17.1	66.0	64.1	22.8	36.7	13.7	17.5	26.1	8.8

Phaseolus vulgaris (*10 days*).—Marked increases in ammonia and residual nitrogen resulted from ammonium nutrition. Although considerable amides accumulated, curiously, ammonium absorption did not materially increase this fraction due apparently to an unfavorable carbohydrate-nitrogen balance in which the high concentration of sugars associated with slow absorption of ammonium favored protein regeneration or synthesis. Ammonium absorption had practically no effect on the amide fraction in the ammonium injured seedlings (22 days). The conditions existing in these seedlings were somewhat analogous to those in the yellow lupines (9 days). An insufficient supply of sugars, even though much ammonia had accumulated, failed to result in amide synthesis, perhaps owing to lack of oxidation products of sugars. Considerable accumulation of residual nitrogen in both series suggests serious need of investigating this fraction.

On the 18th day, part of the cotyledons had fallen from the stems and at this time those still intact were removed and sampled. In both these and the 10-day cotyledons, ammonium tended to interfere with the utilization of starch as shown by the following data (mg. of starch in cotyledons per hundred seedlings): Ten days: NH_4 cotyledons 307 mg., control 293 mg.; eighteen days: NH_4 cotyledons 156 mg., control 144 mg. During the 10- to 18-day interval ammonium hindered utilization of reserve proteins.

Lupinus albus (*17 days*).—Ammonium injury was characterized by transparency followed by flaccidity of a region near the lower part of the hypocotyl. The roots of both series were apparently uninjured. Accumulation of amides in stems and roots of *L. albus,* which is favored by ammonium, distinctly differentiates these seedlings from injured *Phaseolus* (22 days). The conditions in white lupine with ammonium injury closely resembled those in yellow lupine with respect to the concentration of ammonia and amides. The higher concentration of reducing sugars associated with lower concentration of ammonia in ammonium injured *Lupinus albus* stems and roots as compared with ammonium injured *Phaseolus* indicated more favorable conditions for accumulation of amides in the former.

Lupinus luteus (*9 days*).—The type of ammonium injury especially peculiar to this species was characterized by a transverse rupture soon followed by discoloration and flaccidity of a region near the middle of the hypocotyl. In contrast to the white lupine, protein regeneration and formation of amides in stems and roots were hindered by ammonium. The increase of amides in cotyledons was associated with a higher concentration of reducing sugar and a lower concentration of ammonia than in stems and roots. Utilization of reserve protein was slightly hindered, while utilization and regeneration of insoluble acid hydrolyzable polysaccharides were seriously hindered by ammonium nutrition.

TABLE II

CORRELATIONS REGARDING RATES OF ABSORPTION AND UTILIZATION OF AMMONIUM NITROGEN (CALCULATED ON BASIS OF 100 GM. OF DRY KERNELS)

	NH_4-NITROGEN ABSORBED (A)	NH_4-NITROGEN ACCUMULATED (B)	ABSORBED NH_4-NITROGEN UTILIZED (A − B)	EFFICIENCY OF UTILIZATION (A − B)/(A) × 100
	gm.	*gm.*	*gm.*	%
Cucurbita pepo 10 days	1.551	0.873	0.678	43.7
Phaseolus vulgaris 10 days	0.357	0.041	0.316	88.6
Phaseolus vulgaris 22 days	0.642	0.257	0.385	59.8
Phaseolus vulgaris 10–22 days	0.285	0.216	0.069	24.2
Lupinus albus 17 days	0.730	0.308	0.422	57.8
Lupinus luteus 9 days	0.253	0.264	−0.011	.....

By correlating the results in table II with the composition of the kernels of the various species, it appeared that the nature and amount of the food reserves in the seed were important factors in determining the rate of ammonium absorption. The most rapid absorption was associated with *Cucurbita* (high oil reserve), while the lowest rate was associated with the high protein reserve of yellow lupine. Even though *Phaseolus* contained the lowest protein reserve, it did not favor rapid ammonium absorption. White lupine absorbed ammonium more rapidly than *Phaseolus* even though the former contained more protein reserve. Hence the nature of the non-nitrogenous food reserve appeared to be a decisive factor in governing ammonium absorption. Furthermore, the rate of absorption depended upon the stage of growth (state of nutrition) as shown by the 10-day and 10–22-day *Phaseolus*.

In the early stage of growth *Phaseolus* (10-day) accumulated but little ammonia through ammonium absorption, quite in contrast to *Cucurbita*. In the latter stage (10–22-days), as reserves were exhausted an amount of ammonia had accumulated comparable to that shown by *Lupinus luteus*. Five times as much ammonia accumulated as a result of ammonium absorption during the latter 12 days than during the first 10 days, owing to depletion of carbohydrate reserves.

The 17-day white lupine resembled 22-day *Phaseolus* in regard to efficiency of utilizing ammonium. In these seedlings, and especially in yel-

low lupine, ammonium nutrition seriously disrupted the formation and regeneration of organic nitrogen, this condition being associated with low non-nitrogenous food reserves.

Aside from or associated with the type and amount of food reserves, other peculiarities inherent in the various species may have altered the response of the seedlings to ammonium nutrition, and only through more extensive studies can certain generalizations be established.

Summary

1. Etiolated seedlings of *Cucurbita pepo, Phaseolus vulgaris, Lupinus albus,* and *L. luteus,* grown in complete nutrient solutions, were studied with respect to their response to ammonium nutrition as influenced by the type and amount of food reserves.

2. In this investigation the conditions which resulted during ammonium nutrition cannot be ascribed to physiological acidity. It is unfortunate that PRIANISCHNIKOW did not employ complete nutrient solutions.

3. The rates of absorption and utilization of ammonium per hundred grams of dry kernels were apparently dependent upon the type and amount of non-nitrogenous reserves and varied with the stage of growth. The efficiency of the various species with respect to the utilization of absorbed ammonium varied considerably.

4. Ammonium nutrition interfered with the utilization of reserve proteins and non-nitrogenous reserves. Protein regeneration or formation favored by ammonium did not take place at the expense of reserve proteins. Starved *Phaseolus* seedlings resembled those of *Lupinus luteus* in that protein regeneration was hindered by ammonium.

5. Little evidence was obtained in favor of the view emphasized by PRIANISCHNIKOW that amides serve as efficient detoxicants of ammonia.

6. The resistance of seedlings to ammonium injury was apparently governed by the type and amount of non-nitrogenous reserves in the seed. The type of ammonium injury was definitely different in the various species employed.

7. Considerable accumulation of ammonia and low concentration of reducing sugars were associated with ammonium injury which may or may not have been caused by these conditions. There is need of more extensive organo-chemical investigation supplemented with histological and physiochemical studies to determine more satisfactorily the conditions associated with ammonium injury.

8. Further investigation of these and other species is necessary to establish certain generalizations before attempting to classify seedlings with respect to their responses to ammonium salts.

The writer wishes to express his sincere appreciation to Dr. T. G. PHILLIPS whose continued interest and kind advice aided this investigation.

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PRODUCTION OF ALCOHOL AND ACETALDEHYDE BY TOMATOES[1]

FELIX G. GUSTAFSON

Introduction

Ever since the time of PASTEUR it has been known that under some conditions seed plants produce small quantities of alcohol. LECHARTIER and BELLAMY (**10**) were the first to show that fruits, when deprived of air, produce alcohol as well as CO_2. These workers studied a number of varieties of fruits and always found some alcohol produced. They inclosed their fruits in tight containers for several months and in many cases the fruits were considerably disintegrated although they were sterile. From his extensive studies on the maturing of fleshy fruits, GERBER (**3**) came to the conclusion that in many fruits alcohol is formed during the process of ripening, together with volatile acids and esters. MÜLLER-THURGAU and OSTERWALDER (**13**) found alcohol in pears. THOMAS (**18**) found alcohol in apples. Although GERBER studied apples he does not mention having found alcohol in these fruits. ONSLOW and BARKER (**17**) found alcohol in oranges. The concentration was particularly high when the fruits had been stored in an atmosphere high in CO_2, but even in ordinary air alcohol was present to the extent of about 0.03 to 0.10 per cent.

It is thus evident that alcohol is produced in fruits when they are surrounded by air. Under anaerobic conditions alcohol has been found in many plant structures, and reference to much literature on the subject is to be found in the monographs (**8**) on plant respiration.

It has been shown by a number of investigators that acetaldehyde is formed in plants. MAZÉ (**11**) found acetaldehyde in unripe seeds of corn and pear. This acetaldehyde he believed prevented them from germinating. In 1912 KOSTYTSCHEW (**7**) demonstrated that acetaldehyde was produced in the alcoholic fermentation of yeast. His method of demonstrating acetaldehyde consisted in adding zinc chloride to the fermenting liquid, which brought about polymerization of the acetaldehyde, preventing it from being reduced to alcohol. KOSTYTSCHEW *et al.* (**9**) found acetaldehyde in poplar blossoms. Acetaldehyde has also been found in other plants: by MÜLLER-THURGAU and OSTERWALDER (**13**) in pears and apples; by NEUBERG and REINFURTH (**14**) in alcoholic fermentation; by NEUBERG and GOTTSCHALK (**15**) in crushed peas and bananas; by THOMAS (**18**) in apples; by BODNAR and coworkers (**1**) in whole seeds of peas; by KLEIN and PIRSCHLE (**6**) in flowers, leaves, seedlings, and seeds of a number of different species of plants; by HARLEY and FISHER (**5**) in ripe pears.

[1] Paper from the Department of Botany of the University of Michigan, no. 400.

That acetaldehyde is an intermediate compound in anaerobic respiration seems to have been first shown by KOSTYTSCHEW (7), although NEUBERG is given all of the credit. He certainly demonstrated beyond a doubt that acetaldehyde is one of the intermediate products formed during alcoholic fermentation. NEUBERG and REINFURTH by their "Abfangsmethode" have been able to show that acetaldehyde is produced in large quantities during alcoholic fermentation. Their first method consisted of adding sodium bisulphite to the fermenting liquid; the bisulphite united with the acetaldehyde and thus prevented it from being reduced to alcohol. In 1920 they published a second method of binding the acetaldehyde. In this method one molecule of acetaldehyde united with two molecules of dimethylhydroresorcin (dimedon). After the fermentation had been completed the acetaldehyde was quantitatively determined.

Investigation

The work reported in this paper was undertaken as a further investigation of respiration in tomato fruits. The fruits used were picked from the vines as needed, thus insuring freshness of the material.

Both alcohol and acetaldehyde were determined in the fruits and leaves as they came from the plants and also after the fruits had been respiring anaerobically for various lengths of time. Nitrogen was used in most of the experiments to replace air, although a few experiments were conducted in which CO_2 replaced air. In four experiments the fruits were placed in a large container, which was then closed, and as respiration proceeded the oxygen was used up and replaced with CO_2.

When either CO_2 or nitrogen was used to replace the air, these gases were passed through wash bottles containing chromous chloride, to remove all oxygen. The gas was then passed through the chamber containing the fruits. In the experiments on alcohol production fruits were in a nitrogen atmosphere for 24, 48, and 72 hours. For these experiments a quantity of fruits was collected and divided into four groups, each group having fruits of the same ripeness and size. Only uninjured fruits were used. One lot of fruits was analyzed at once, and the others were placed in separate containers through which nitrogen was passed. One lot was analyzed each day. In this way it was possible to study the progressive accumulation of alcohol in similar fruits.

Several methods of determining alcohol were tried. In the beginning a method of steam distillation was used. A weighed quantity was taken and the fruits cut into small pieces, placed in a 5-liter round-bottom flask and steam-distilled for 5 to 6 hours, or until about 600 cc. of distillate had been collected. The distillate was collected in two receiver flasks, each containing 50 cc. distilled water at the beginning, and surrounded by ice. The

total distillate was made up to 760 cc. each time by addition of distilled water, and 500 cc. of this were used for the alcohol determination and 260 cc. for the acetaldehyde determination.

Acetaldehyde was determined according to the method of NEUBERG and GOTTSCHALK (**16**). For the acetaldehyde determination 260 cc. of the distillate were used. To this 40 cc. of hydroxylamine sulphate (1.0 per cent.) were added and the mixture permitted to stand for an hour. The H_2SO_4 liberated was titrated with 0.1 N NaOH, using methyl orange as indicator. To insure that the same end point was always reached, two buffer flasks with pH 3.6 and 5.8 were used, each having the same amount of liquid as the titration flask. A correction was made for the neutralizing effect of distilled water. From several titrations of laboratory distilled water it was found that the addition of 100 cc. of distilled water was equivalent to 0.2 cc. 0.1 N NaOH. NEUBERG and GOTTSCHALK give the conversion factor as 1 cc. 0.1 N NaOH equivalent to 0.0044 gm. acetaldehyde.

The alcohol and acetaldehyde were oxidized to acetic acid with potassium dichromate and sulphuric acid. This was then distilled from a claison flask and the acetic acid titrated with 0.1 N NaOH, using phenolphthalein as indicator. By calculation the total alcohol in the 760 cc. of distillate was found. The acetic acid formed from the aldehyde was of course subtracted from the total in making this calculation. This method of determining alcohol gave very high values, and as other methods gave only about half as high a percentage these figures are not presented. The high concentration of alcohol is probably due to the long distillation at steam temperature. This probably distilled over some other substances, such as esters, in addition to alcohol and acetaldehyde, which later reacted with the NaOH in the titration.

If this method of the distillation vitiated the alcohol determination it did the same to the acetaldehyde analysis. The acetaldehyde analyses are not presented because of their lack of reliability. All one can say is that according to the determinations following the NEUBERG-GOTTSCHALK method, acetaldehyde was always found to be present. How much of this apparent acetaldehyde was due to acids that may have distilled over is not known. There was very little variation in the acetaldehyde noted, and there was no accumulation as the time of anaerobiosis was continued. The second method of alcohol determination did not lend itself readily to acetaldehyde analysis and it was therefore not repeated.

The alcohol was finally determined according to the method used by CANNAN and SULZER (**2**) in determining alcohol in blood. The apparatus used was modified to utilize more material than these investigators used. In these experiments a known amount (about 300 gm.) of freshly ground tomato pulp was placed in a 2-liter round-bottom flask, and between 500

and 600 gm. of anhydrous Na_2SO_4 added to prevent frothing. Distillation was carried out at a reduced pressure, which was around 6 cm. of mercury for all experiments, into two tubes 2 × 25 cm., each containing 35 cc. of concentrated H_2SO_4. At the pressure of 6 cm. mercury bubbling or boiling was produced at 40° C. In no experiment did the temperature rise as high as 45° C. After several preliminary experiments the time of distillation was placed at three hours. The H_2SO_4 from both receiving tubes was brought together and the volume made up to 100 cc. A 20-cc. portion of this H_2SO_4 was added drop by drop to a known quantity of 0.2 N $K_2Cr_2O_7$, which quantity varied with the experiment. The dichromate flask was kept cool by immersion in running water while the sulphuric acid was added. The oxidizing mixture of sulphuric acid and potassium dichromate was allowed to stand for one hour to complete the oxidation of the alcohol to acetic acid. This solution was then diluted until the sulphuric acid was 5 per cent., and an excess of 10 per cent. KI added (about 10 cc.). The iodine liberated by the excess dichromate was titrated with 0.1 N sodium thiosulphate, using starch as the indicator. In this determination 1 cc. 0.1 N $K_2Cr_2O_7$ is equivalent to 1.15 mg. of alcohol.

With the CANNAN-SULZER method much more complete and better organized experiments were conducted than with the first mentioned method. For this reason, and also because it is a more reliable method, the results obtained with it are presented in table I.

Table I shows that there is considerable alcohol in the fruits that have been in air; that this is at its maximum in the nearly ripe orange-red fruits; and that in the green fruits the amount of alcohol decreases as the fruit size is decreased, even though the small young fruits respire very much more than the larger fruits. During these investigations an experiment was conducted having as its purpose to study the relation between size and alcohol production. For this experiment orange colored tomato fruits were chosen, because among the ripening fruits they respire rapidly and for the experiment it was necessary to have fruits of the same physiological age. In one lot the fruits were 10.0 cm. in diameter and in the other lot only 3.5 cm. in diameter. Analysis was made directly after the fruits were removed from the vines. The large fruits had an alcohol content of 0.012 per cent. while in the smaller fruits only 0.0081 per cent. alcohol was found. From this experiment as well as from the experiments noted in table I it seems evident that when respiration is rapid the alcohol formation (*i.e.*, the anaerobic respiration) is greater in the large fruits than in the smaller ones.

Table I also shows that alcohol, unlike acetaldehyde, accumulates as the time of anaerobiosis lengthens; and that the alcohol increase is greatest in those fruits carrying on the most rapid respiration, namely, the small green fruits, in which the initial alcohol content was by far the smallest. There

TABLE I

PERCENTAGE OF ALCOHOL IN TOMATO FRUITS

CONDITION OF FRUITS	DIRECTLY FROM PLANT	NUMBER OF HOURS IN NITROGEN		
		24	48	72
	%	%	%	%
Red-ripe	0.0125	0.04	0.065	0.071
	0.0140	0.031	0.057	
	(1)*0.0144	(1)0.031	(1)0.054	(1)0.070
			0.054	
Orange-red	0.023	(2)0.0255	(2)0.0555	(2)0.0315
	(3)0.025	(3)0.028	(3)0.061	(3)0.046
Orange	(4)0.016	(4)0.037	(4)0.040	(4)0.062
Pink-orange	.....	.....	0.041 0.035	
Pink	0.012	(5)0.016	(5)0.034	
	(6)0.016	(6)0.017	(6)0.096†	(6)0.042
Green	0.016	(7)0.021	(7)0.047	(7)0.094
Large	0.014			
Medium	(8)0.012	(8)0.039	(8)0.077	(8)0.133
Small	(9)0.008	(9)0.066	(9)0.116	(9)0.150
2 cm. diameter	(10)0.0015	(10)0.003	(10)0.095	(10)0.185
1–2 cm. "	0.0011	(11)0.104	(11)0.135	(11)0.205

* Similar numbers in the brackets before the figures indicate that these fruits were picked at the same time and were all alike, but have been in nitrogen different lengths of time.

† Fruit very soft when taken out of nitrogen.

is one exception to the preceding statement. In the orange-red fruits there is a decrease in amount of alcohol from 48 hours to 72 hours. There is no apparent reason for this decrease.

TABLE II

PERCENTAGE OF ALCOHOL IN OTHER PLANT STRUCTURES

MATERIAL	IN AIR	IN NITROGEN 48 HOURS
Potato		
Old	0.0013	0.019
New	0.0012	.....
Peas germinated 48 hours	0.037	0.244
Tomato leaves	0.0031	.....

It should be pointed out that although table I is headed alcohol yet it actually applies to alcohol plus acetaldehyde. Both are oxidized by the sulphuric acid and the potassium dichromate. The amount of acetaldehyde, however, is a very small part of the total volatile material as given in table I.

Table II presents a miscellaneous collection and is presented only for the sake of comparison. It is usually stated in works on respiration (**8**) that potatoes do not produce any alcohol, and that germinating peas produce large quantities of it. From table II one must conclude that some alcohol is produced in potatoes although in small quantity, and that this quantity increases only very slightly when the tubers are completely deprived of oxygen for 48 hours. Peas germinating in air produce somewhat more alcohol than orange-red tomatoes, and the amount of alcohol increases very much when the peas are deprived of oxygen for a period of 48 hours. The alcohol content in tomato leaves directly from the vines is intermediate between that from tomato fruits with a diameter of 2 cm. and that from fruits with a diameter of 4 to 5 cm.

Determination of acetaldehyde depends upon titration with a base, and it is obvious that any acid which had distilled over would increase the amount of hydroxide needed to bring about neutralization and this would increase the apparent amount of acetaldehyde. Also other alcohols than ethyl alcohol would be oxidized by the H_2SO_4 and $K_2Cr_2O_7$ and the apparent amount of ethyl alcohol would thereby be increased. Therefore it was deemed appropriate to make qualitative tests for acids, other alcohols, and aldehydes to discover whether the figures just cited could be considered to represent the ethyl alcohol as stated in the tables.

Not only acids and alcohols were tested for but also other compounds with a boiling point near 100° C. and known to be associated with anaerobic respiration. These compounds were: formic and acetic acids, formaldehyde, acetaldehyde and higher aldehydes, methyl, ethyl, n. propyl, isopropyl, isoamyl and isobutyl alcohols, acetone, acrolein, and methyl glyoxal. Of these acetaldehyde, ethyl alcohol, and a trace of acids were always found. In several experiments there was a trace of methyl alcohol. In one experiment each formic and acetic acids and formaldehyde were found as a trace. The other compounds were always absent. The experimental material consisted of fresh leaves, large green fruits, also small green, large green, faintly pink or yellow, and red ripe fruits, which had been respiring anaerobically from 48 to 96 hours in nitrogen. This material includes all that was used in the quantitative analyses.

From the results of these qualitative analyses it seems fairly certain that the only aldehyde found extensively is acetaldehyde, and only ethyl alcohol is formed; and it can then be concluded that in the respiration of tomatoes

(both when in air and when deprived of oxygen) acetaldehyde and alcohol are formed. Since acetaldehyde does not accumulate as the time of anaerobiosis lengthens, it is evident that it is not a final product of anaerobic respiration. On the other hand alcohol does accumulate and is therefore a final product of anaerobiosis.

The experiments bring out the fact that in the larger fruits there is a certain amount of anaerobic respiration even when the fruits are surrounded by air. In a previous paper the writer (**4**) pointed out that, from the behavior of the fruits when deprived of oxygen, it seemed very likely that there was some anaerobic respiration in tomato fruits even when in air, and these experiments prove that this assumption was correct. The experiments further show that the anaerobic respiration when the fruits are in air is much greater in large than in small fruits of the same physiological age. At least the alcoholic formation is greater in the large fruits than in the small ones, when both fruits are orange in color.

As yet no analyses have been made of the gas from the interior of tomato fruits, but the CO_2 content is probably high and the oxygen low. This is to be expected from other work, as that of MAGNESS (**12**) on apples and potatoes and some unpublished analyses of the gas from cacti by the writer. According to KOSTYTSCHEW and others, the preliminary steps in all types of respiration are the same. If that is true there is no reason why the aerobic and anaerobic respirations should not take place at the same time in a fruit, when the oxygen concentration becomes low. Some molecules of the intermediate compounds may follow the aerobic respiration path leading to the CO_2 and H_2O formation, while others may be changed to CO_2 and C_2H_5OH. The ratio between the two types of respiration would vary with the amount of oxygen present. As the oxygen became depleted the anaerobic type would increase, until finally all of the respiration was of that kind.

In his work on apples, THOMAS (**18**) distinguishes two types of respiration in which alcohol is formed. In the absence of oxygen there is little acetaldehyde formed with the alcohol. This type he calls anaerobic zymasis. The other type which he calls carbon dioxide-zymasis takes place in a high concentration of CO_2 in the presence of oxygen. Under this condition a high percentage of acetaldehyde is formed together with the alcohol. In the experiments on tomatoes there is no such distinction. THOMAS reports that in carbon dioxide-zymasis the acetaldehyde alcohol ratio was near one to two. There is no essential difference in this ratio in the absence of oxygen and in its presence, in the work of the tomato fruits. The writer's experiments on carbon dioxide-zymasis were not so extensive as those of THOMAS, but nevertheless it seems that there is a decided difference in the behavior of apples and tomatoes.

Summary

1. Acetaldehyde has been found in all tomato fruits, under all conditions of treatment. The amount of this aldehyde does not increase as the time of anaerobiosis lengthens.

2. Ethyl alcohol has also been found in all tomato fruits, under all conditions of treatment. The amount of alcohol increases with the length of anaerobiosis.

3. Fruits as taken off the vines, which had been in the greenhouse under natural conditions, contained some acetaldehyde and alcohol, although not so much as in fruits deprived of oxygen.

4. Large fruits contained more alcohol than small fruits when analyzed directly from the vines.

5. In qualitative experiments, compounds known to be associated with anaerobic respiration and having a boiling point near 100° C. were tested for. Acetaldehyde and ethyl alcohol were always found. Traces of methyl alcohol were found in several experiments, and traces of formic and acetic acids and formaldehyde were each found in only one experiment.

Acknowledgment is made of a grant from the Faculty Research Fund of the University of Michigan for most of the analytical work, which was done by Miss Edna Warweg and Mr. Sam Granick.

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COMPARISON OF THE HEATING AND FREEZING METHODS OF KILLING PLANT MATERIAL FOR CRYOSCOPIC DETERMINATIONS

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In a study of the changes in the sap concentration of *Larrea tridentata* reported by the writer (**1**), the leaf and twig tissues were killed by heating them in closed vessels in boiling water for a period of 30 minutes before the sap was extracted for the cryoscopic determinations. This is a new departure in cryoscopic studies in this country, where plant tissues have been killed by subjecting them to freezing temperatures. The heating method, however, has been used extensively by WALTER (**3**). The method is described in detail in the paper just cited (**1**).

On theoretical grounds the heating method of rendering plant tissues permeable to the cell contents is open to criticism. Both enzymatic changes and other chemical reactions may be speeded up two to three times for every 10° C. rise in temperature; that is, the hydrolysis of sugars and other cell contents may occur which would change the osmotic concentration of the cell sap from its natural condition. In order to establish the practical importance of such changes in relation to a determination of the Ov of any particular species, comparative studies were made between the heating and freezing methods. The purpose of this paper is to report the results of these experiments.

In cooperation with Dr. MATLOCK and Mr. HOBART of the agronomy department of the University of Arizona, a comparative study was conducted using field cotton. This experiment also served the purpose of comparing results obtained by the use of a Beckmann thermometer with those found when a Drucker-Burian thermometer was employed. The material used for this experiment was cotton grown on experimental plots on the university farm near Tucson. Two sets of leaf samples were collected simultaneously from each of two plots. Each plot was divided roughly into four sections, each set of samples consisting of one sample from each section and each sample containing about twelve leaves picked at random from plants within a given section. One set of samples from each plot was collected by MATLOCK and HOBART, each collecting two samples, while the other two sets were collected by the writer. One set of samples from each plot was frozen in an ice-salt mixture and one set from each plot was heated in boiling water for 30 minutes. All of the osmotic value determinations for plot no. 7 were made by MATLOCK and HOBART, using a Beckmann thermometer, and they also made the determinations for the set of samples which they collected on plot no. 6. The osmotic values for the other set of samples

from plot no. 6 were determined by the writer, using a Drucker-Burian thermometer. The results of this experiment are given in table I.

TABLE I

COMPARISON OF THE FREEZING AND BOILING METHODS OF KILLING COTTON LEAF TISSUES FOR SAP EXTRACTION IN CRYOSCOPIC DETERMINATIONS

PLOT	SECTION	FROZEN Δ	HEATED Δ	FROZEN Ov	HEATED Ov	Δ OF HEATED − Δ OF FROZEN MATERIAL	Ov OF DIFFERENCE
7	1	0.940	1.120	11.320	13.480	0.180	2.171
	2	1.016	1.102	12.232	13.264	0.086	1.037
	3	0.934	0.992	11.248	11.944	0.058	0.699
	4	1.133	1.201	13.636	14.452	0.068	0.820
Average	.....	1.006	1.104	.....	.....	0.097	1.181
6	1	1.056	1.111	12.712	13.372	0.055	0.663
	2	1.395	1.007	16.780	12.124	−0.338	4.676
	3	0.848	1.047	10.216	12.604	0.199	2.399
	4	1.562	1.313	18.784	15.796	−0.249	3.001
Average	.....	1.215	1.120	.....	.....	−0.223	2.688

It will be noted that for plot no. 7, all determinations for which were made with a Beckmann thermometer by the same operators, the heated material yielded a higher osmotic value in each case than did the frozen material. This fact would tend to support the theory that hydrolysis or enzymatic action took place in the heated material. However, the difference between the depressions of the freezing point found in the frozen material for sections 3 and 4 of plot no. 7 is 0.199°, which is equal to 2.399 atmospheres, and this could be taken as an indication that differences in the values are attributable to variations in the material and lack of uniformity in the sampling process. On the other hand, this is somewhat offset by the fact that the differences between sections 3 and 4 of plot no. 7 for the heated material are practically the same and are in the same order as those for the frozen tissue.

When the results for plot no. 6 are compared, greater differences and much less uniformity are apparent. This is somewhat to be expected, since the determinations were made by different operators using different type thermometers. However, in this case the values found for the heated material are not uniformly higher than those of the frozen material. Such varia-

tions cannot be ascribed entirely to the method of killing, since changes brought about by raising the temperature of the tissues would, in the majority of plant tissues at least, increase the osmotic concentration of the sap. The difference between the highest and lowest values for the frozen material is a little over 0.7° C., while the highest and lowest values for the heated material show a difference of a little over 0.3° C. Again these facts would tend to show that differences between the values for the frozen and heated material are due to actual differences in the samples, and not to the method of killing or of determining the depressions of the freezing point. It would seem, therefore, that the results of this experiment are not against the use of the heating method of killing tissue for juice extraction in cryoscopic determinations, even with a succulent, rapidly growing plant like cotton, with large leaves in which the amount of hydrolyzable material is probably much greater than in the more slowly growing, non-succulent, small leaved *Larrea*.

WALTER (3) presents some comparative data on values obtained for living tissue (L), tissue killed by heating in water at 100° C. (H), and tissue killed by freezing with liquid nitrogen (K). Several species of plants were used. For leaves of *Vitis* he found the following values: L = 7.47, H = 10.48, K = 10.00 atmospheres; for *Parictana:* L = 7.47, H = 10.61, K = 11.25 atmospheres. The sap expressed from *Buxus sempervirens,* a thick heavy leaved plant, gave values: L = 7.16, H = 25.64 and 25.49, and K = 25.95 and 26.74 atmospheres. In the case of *Aucuba japonica* the method of killing the tissue seems not to be important, for three samples of leaf tissue killed by heating at 100° C. gave osmotic values for their expressed sap of 21.28, 20.50, and 21.08 atmospheres (average = 20.96 atms.), while three samples killed by freezing with liquid nitrogen gave values of 21.94, 22.00, and 19.77 atmospheres (average = 21.30). *Ficus elastica* also gave values which were very close for heated and frozen material, the values for the former being 9.73 and 9.68 atmospheres and those for the latter 9.90 and 9.67 atmospheres.

Determinations of the osmotic values of small whole plants taken from their natural environment showed, in the work of WALTER, no distinct influence of the method used in killing the tissues before expressing the sap. The results of this experiment were as follows:

Thymus serpyllum	H = 12.03	and	12.56	atmospheres
	K = 10.23	"	13.02	"
Allyssum montanum	H = 14.00	"	14.84	"
	K = 14.10	"	12.64	"
Pirola umbellata	H = 21.09	"	K = 19.86	"

Experiments with *Prunus laurocerasus* resulted in increasingly higher osmotic values for the cell sap as the period of killing the tissues by heating

was lengthened, and when the heating was continued long enough completely to kill all of the cells, H = 25.09 and 25.13 atmospheres, as compared with the values for K which were 22.36 and 22.26 atmospheres. These results were attributed to the fact that *Prunus* contains relatively large quantities of easily hydrolyzable glucosides, which upon hydrolysis increase the osmotic concentration of the cell sap. When working with such plants or plant tissue, if very exact determinations are required the method of killing by freezing should be employed.

Further to test the reliability of the heating method, a comparison with the freezing method was made using leaves and small twigs of *Larrea tridentata*. For this experiment samples of leaves and twigs were collected from *Larrea* bushes near the main laboratory building. The first collection consisted of twelve samples from bush A, eight from bush B, and four each from bushes C, D, E, and F. The second collection, made 4 days later, included four samples each from the same bushes except bush D and from three additional bushes, G, H, and I. The samples were all taken from the bushes in rapid succession, and immediately the samples from each bush were divided into two lots. One of these was subjected to the heating method of killing, the other to the freezing method.

The freezing of the plant tissues was accomplished with solid carbon dioxide (dry ice), which gives a temperature of about −180° C. The samples were in glass test tube-like vessels with cork stoppers which fit compactly, in aluminum canisters with screw tops. These canisters were completely buried in dry ice in a calorimeter tank, which in turn was lowered into a 5-gallon crock and completely covered with towels. The samples from the first collection were left in the freezing chamber 50 minutes, 30 minutes, and 12 hours, as indicated in table II, which also gives the osmotic values obtained for these samples.

In eleven of the eighteen comparisons shown in table II, the values for the heated plant materials were greater (>) than those found for the frozen tissues. In five instances the compared values were equal or nearly so, and the samples from bush E gave values for the heated materials which were less (<) than those for the frozen samples, although the differences were less than one atmosphere.

These results lend some support to the possibility that the heating process may produce significant changes in the concentration of the plant sap. In only three instances, however, was the difference between the values for the heated and frozen material greater than two atmospheres, and in but eight of the eighteen comparisons was the difference more than one atmosphere. While these differences are greater than desirable, they are partly attributable to chance pairing. For example, in the case of bush B, samples 22 and 23, the difference is 3.05 atmospheres. If sample

TABLE II

OSMOTIC VALUES OBTAINED FOR COMPARABLE SAMPLES (FIRST COLLECTION) OF LEAVES AND TWIGS OF *LARREA TRIDENTATA* BY HEATING AND BY FREEZING METHODS OF KILLING THE TISSUES PRIOR TO EXPRESSING SAP

Bush	Heated tissues			Comparison of Ov's	Frozen tissues			Difference in Ov's
	Sample no.	Ov	Duration of treatment		Sample no.	Ov	Duration of treatment	
			min.				*min.*	
A	12	28.17	30	>	13	26.37	50	1.80
"	14	29.06	"	>	15	26.55	"	2.51
"	16	28.11	"	>	17	27.03	"	1.08
"	18	27.75	"	>	19	27.11	"	0.64
B	20	30.20	"	=	21	30.26	"	0.06
"	22	31.99	"	>	23	28.94	"	3.05
"	24	29.90	"	=	25	29.90	"	0.00
"	26	30.98	"	>	27	28.86	"	2.12
C	28	28.94	"	=	29	28.70	30	0.24
"	30	29.30	"	>	31	28.29	"	1.01
D	32	41.12	"	>	33	39.57	"	1.55
"	34	40.76	"	>	35	40.05	"	0.71
E	36	34.83	"	<	37	35.75	"	0.92
"	38	33.78	"	<	39	34.56	"	0.78
F	40	28.58	"	=	41	28.58	"	0.00
"	42	27.93	"	=	42	28.11	"	0.18
							hr.	
A	38×	26.49	"	>	37×	25.05	12	1.44
"	40×	26.55	"	>	39×	25.11	"	1.44

22 had been paired with sample 21 the difference would have been only 1.73 atmospheres. It will be noticed also that some of the heated samples, 22 and 24 for example, exhibited as great differences as 2.09 atmospheres.

The average of the first four values obtained for the heated material from bush A is 28.25 atmospheres and for the frozen material 26.76 atmospheres. The difference between these average values is but 1.49 atmospheres. The average value for the heated tissues from bush B is 30.77 atmospheres and for the frozen samples from B, 29.49 atmospheres, making a difference of only 1.25 atmospheres. Using the differences between the average osmotic values found for bushes A and B for comparison, instead of the paired sample differences, leaves only one case in the entire first collection in which the heated tissues yielded a value more than 1.50 atmospheres higher than that for the frozen material.

Previous investigations with *Larrea* have shown that a difference of as much as 0.5 atmospheres may be attributed to variations in the samples

from the same bush. If this amount be subtracted from the comparative difference, only about 1 atmosphere increase in the osmotic value of the sap need be attributed to the heating process.

TABLE III

OSMOTIC VALUES OBTAINED FOR *LARREA* FROM THE SECOND COLLECTION. FOURTEEN SAMPLES WERE HEATED 30 MINUTES, TEN WERE FROZEN 12 HOURS, AND FOUR WERE UNTREATED PRIOR TO EXTRACTING THE SAP

BUSH	HEATED TISSUES		COMPARISON OF Ov's	FROZEN TISSUES		UNTREATED TISSUES		DIFFERENCES IN Ov's
	SAMPLE NO.	Ov		SAMPLE NO.	Ov	SAMPLE NO.	Ov	
A	1×	24.10	<	.....	.....	2×	27.57	3.47
"	7	23.74	<	9	26.67	.....	.....	2.93
B	10	29.00	<	.....	.....	28	31.27	2.27
"	29	29.48	<	30	29.90	.....	.....	0.42
C	48	28.05	<	.....	.....	5	32.05	4.00
"	35	27.27	<	36	30.54	.....	.....	3.27
E	20	23.38	<	.....	.....	21	25.77	2.39
"	22	23.38	<	23	27.15	.....	.....	3.77
G	11	38.79	<	17	42.31	.....	.....	3.52
"	18	38.56	<	19	43.68	.....	.....	5.12
H	31	27.09	<	32	30.98	.....	.....	3.89
"	33	27.69	<	34	31.51	.....	.....	3.82
I	37	27.63	<	38	29.48	.....	.....	1.85
"	39	27.69	<	40	28.17	.....	.....	0.48

The data presented in table III for the second collection of *Larrea* leaves and twigs completely reverse the evidence in regard to the heating and freezing method of killing plant material for cryoscopic determinations of its sap concentration. In this series of determinations the heated samples gave osmotic values which were, in every case, less than those found for the frozen and the untreated tissues.

This astonishing difference in results can probably be attributed almost entirely to the fact that, while the determinations of the Ov for the first collection were made the following day, it was necessary to store the second collection of samples nearly three weeks before the osmotic values were determined. The freezing process did not sterilize the samples as did the heating method, and apparently the storage temperature was not sufficiently low to prevent bacterial and enzymatic activity. The fact that the values for the untreated material more nearly equal the values for the frozen samples than those for the heated tissues, and are in most cases higher than the frozen, helps to bear out the preceding contention.

Summary

1. A comparison of results obtained by using the heating and the freezing methods of killing cotton and creosote (*Larrea*) tissues for cryoscopic determinations of the sap concentration shows that the heating process may increase the concentration as much as one atmosphere in some cases. For an extensive series of determinations over a period of time, however, the heating method gives just as reliable an indication of the changes in sap concentration, of certain plant species at least, as does the more expensive and time-consuming method of freezing the tissues.

2. Not only is the heating method reliable for comparative studies of most species, but it has the added advantage of rendering the plant material capable of storage for considerable periods of time without significant change in its sap concentration. Heated samples have been stored for as long as 176 days at room temperature with an average increase in Ov of only 1.4 atmospheres in samples which exhibited differences of as much as 0.8 atmospheres among themselves.

3. The heating method is readily adaptable to extended collections of samples in the field. The samples may be heated in boiling water over a camp stove, or even a camp fire, immediately after collection, and the osmotic values determined after return to the laboratory, without fear of much, if any, change.

4. With the plant material used in these experiments the duration of freezing apparently made little difference, samples frozen 30 minutes giving approximately the same range of values as those frozen for longer periods. MEYER (**2**) states that a period of at least 8 hours is desirable, and most workers have frozen their material much longer than the 30 minutes required for killing material by heating. This emphasizes the saving of time usually possible by employing the heating technique.

5. The small amount of fuel necessary for killing a group of samples is considerably less expensive than the materials needed for freezing plant material, especially if solid carbon dioxide is used, as is recommended by some workers.

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INFLUENCE OF THYROXIN ON THE GROWTH OF PLANTS

ELIZABETH ELLEN DAVIS

(WITH ONE FIGURE)

Introduction

The general action of thyroxin is probably as a catalyst in the process of combustion, increasing the rate of metabolism. Some work has been done on the effect of thyroxin upon the growth of plants. ABDERHALDEN (**1**) found that alcoholic fermentation is usually accelerated by either synthetic thyroxin or that of natural origin. He noted, however, that at times the fermentation was retarded. These differences he believed due to the variable condition of the yeast cells used. NIETHAMMER (**4**) states: "thyroid extract and zinc sulphate, which are known stimulants for seeds, also activate resting buds and to a slight degree cell division." BOSE (**2**) claims that he obtained a maximum activity in carbon assimilation with thyroid extract at a dilution of one part in a billion. REBELLO (**5**) in his work on white bulbs of hyacinth found an acceleration of growth when he used dry thyroid powder added to water and when he used 0.5 cc. of 1:500 solution of nucleoprotein and thyroglobulin. When he used several decigrams of fresh thyroid gland in water the bulbs developed as well as the controls which were grown in water. BUDDINGTON (**3**), in his experiments on bulbs of *Allium* grown in Pfeffer's solution, discovered that the growth of the root tips was retarded in proportion to the amount of thyroid material used, and that potassium iodide in the same amounts as that in thyroid substance had no effect on the root tips; further, he found that the early leaves were not affected by the presence of thyroxin.

Methods and materials

No workers, so far as the writer has been able to determine, have tried the injection method of treating plants with thyroid material; all have used the thyroid material in nutrient solution or in pure water. The method of injection was chosen so that the thyroxin could not react with anything in the nutrient solution or in the soil. For injection it was necessary to choose plants which have hollow stems or which grow from bulbs. *Vicia faba,* the Windsor bean, fulfils the first requirement, *Allium* and *Narcissus* the second. *Pisum sativum* also was used in these experiments.

A cubic centimeter syringe with a fine hypodermic needle was used to make the injections. For the first part of the experiments on *Allium* and a part of those on *Vicia faba,* desiccated thyroid gland was employed, a 2-grain tablet in 20 cc. of water; but in order to escape the possible effects

of the organic material, the experiments were continued with the sodium salt of thyroxin in concentration of 0.4 mg. to 5 cc. water. This material, obtained from Squibb and Sons, is stated to be three hundred times as concentrated as is the desiccated thyroid gland.

The controls were injected with water, and received exactly the same treatment as the experimental plants. Measurements were taken in centimeters and weight in grams. The bulbs of *Allium* were grown in the greenhouse under uniform conditions of temperature, moisture, and light. These and the *Vicia faba* seeds were planted in sandy loam, and the peas and *Narcissus* were grown in nutrient solution or in water. The procedure in the case of the peas differed from that already described in that the thyroxin was added to the water rather than injected into the plants.

Experimental results

Growth of *Allium* from thyroxin-treated bulbs

Medium sized, solid, unsprouted bulbs were chosen and placed in water so that roots might be well started before the injection was made. The bulbs were then injected with thyroid solution and planted in sandy loam. Preliminary experiments were performed using desiccated thyroid gland in concentration of 2 grains in 20 cc. water. The flower stalks developed first on the treated bulbs. In another experiment using 12 bulbs, 5 cc. of the solution of desiccated thyroid gland were injected. The plants from the treated bulbs were depressed in vegetative growth, varying on different observations from 7.7 to 28 per cent. The height of the tallest leaves was taken as a measure of growth. Flowering occurred sooner in the treated bulbs, with the exception of two sets of treated and controls which did not flower at all. In one set the flowering took place three weeks earlier in the treated bulbs; and in three sets one week earlier.

In the second group of *Allium* the method was the same but more plants were used, and the weights of the roots were recorded so that more conclusive results might be obtained. A solution of the strength 0.4 mg. sodium salt of thyroxin in 5 cc. water was injected into 36 bulbs and water into 36 bulbs. The doses varied from 1 to 5 cc., but there was no real difference in the development of the three series, hence the figures for all are here combined. Considering height as a measure of growth, table I and figure 1 indicate that the treated material showed a decrease until the sixtieth day after planting, at which time they began to gain in height over the controls. On the sixtieth day after planting, all of the leaves of each plant were measured. The treated averaged one leaf less per plant than the controls but the heights of the plants were approximately the same.

The number of flower stalks was slightly greater among the treated plants: six more than the controls at 88 days after planting, and five more

TABLE I

HEIGHTS OF *ALLIUM* PLANTS FROM BULBS TREATED WITH THYROXIN

NUMBER OF DAYS AFTER PLANTING	TREATED		CONTROL		PERCENTAGE DIFFERENCE IN TREATED PLANTS
	NUMBER OF PLANTS	AVERAGE HEIGHT	NUMBER OF PLANTS	AVERAGE HEIGHT	
		cm.		*cm.*	%
32	35	9.93	35	12.06	−17.06*
46	34	26.79	36	37.27	− 1.76
60	34	39.19	36	38.16	+ 2.72
74	34	47.50	33	43.15	+10.10
88	34	49.92	33	46.42	+ 7.50

* Plus sign indicates that the treated showed greater growth than the controls, the minus sign indicates that they exhibited less growth.

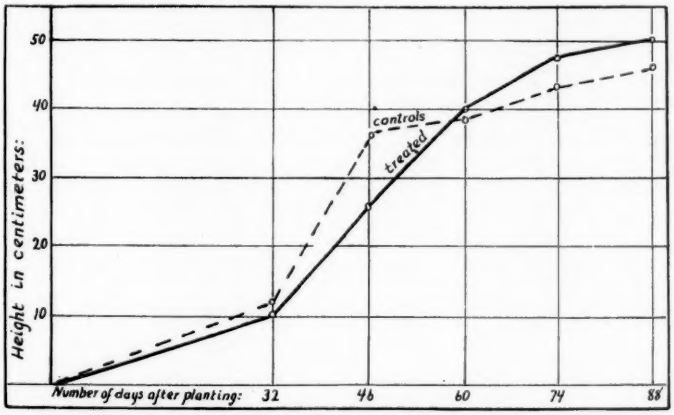

FIG. 1. Heights of *Allium* plants from thyroxin-treated bulbs. The treated plants showed a decrease for the first 60 days but after that they surpassed the controls slightly. The controls had an average of one more leaf per plant.

at 102 days after planting. At the final measurement, 102 days after planting, the treated plants averaged about 34 cm. in height and the controls about 21 cm. The opening of inflorescences took place sooner and progressed more rapidly in the treated than in the controls, as shown in table II. One hundred and sixteen days after planting, the treated showed almost three times as many open inflorescences as the controls.

The roots were cut from the bulbs, washed, and weighed. Table III shows that the average green weight of roots per plant of the treated was 25.87 per cent. less than that of the controls. The weight of the air-dried

TABLE II

OPENING OF INFLORESCENCES OF *ALLIUM* PLANTS FROM BULBS TREATED WITH THYROXIN; 34 EACH OF TREATED PLANTS AND CONTROLS

NUMBER OF DAYS AFTER PLANTING	NUMBER OF INFLORESCENCES OPEN	
	TREATED, 34 PLANTS	CONTROLS, 34 PLANTS
96	2	0
100	6	1
105	8	3
110	9	3
116	17	6
123	20	12
130	20	16
137	21	17

TABLE III

WEIGHT OF ROOTS OF *ALLIUM* PLANTS FROM BULBS TREATED WITH THYROXIN

NUMBER		AVERAGE GREEN WEIGHT		DEPRESSION OF TREATED	AVERAGE DRY WEIGHT		DEPRESSION OF TREATED
TREATED	CONTROL	TREATED	CONTROL		TREATED	CONTROL	
		gm.	*gm.*	%	*gm.*	*gm.*	%
31	32	16.67	22.49	25.87	1.45	1.83	20.76

roots was consistent with the green weight; the treated showed a depression in weight of 20.76 per cent.

These experiments as a whole show that thyroxin does not have any consistent effect on green tops of *Allium*, but has a well marked accelerating influence on the time of flowering and a stunting effect on the roots.

GROWTH OF *NARCISSUS* AND *VICIA FABA* AS INFLUENCED BY THYROXIN

The experiment on *Narcissus* was conducted in the same manner as those on *Allium*, except that the bulbs were grown in water instead of in soil. Seventeen bulbs were injected with 2.5 cc. of solution made by adding 0.4 mg. sodium salt of thyroxin to 5 cc. water. The treated individuals showed a somewhat better vegetative growth and the inflorescence opened sooner. All of the inflorescences of the treated material were open three days before all of the inflorescences of the controls. These results correspond with those found in the case of *Allium*, except for the fact that some of the plants from treated bulbs of *Allium* showed a depression in vegetative growth whereas others showed acceleration.

Vicia faba material was treated by injecting thyroxin solution into the

youngest internode, or the next youngest in case the epidermis broke. Injections were made each week for seven weeks with fresh solutions. It was impossible to determine just how much of the solution remained within the stem, but the minimum was not less than 0.1 cc. In all, 42 plants were studied, 20 grown in the greenhouse and 22 out-of-doors. The results in both cases were approximately the same, although the plants grown out-of-doors were, as would be expected, consistently shorter than those grown in the greenhouse. The treated showed a depression in growth up to 9.99 per cent. Flowering and formation of pods occurred at approximately the same time in the treated and the controls.

Growth of *Pisum sativum* in solutions containing thyroxin

Experiments were carried out to determine whether or not thyroxin has a stunting effect on the growth of peas. Buddington (3) found that the roots of onions grown in Pfeffer's solution with thyroxin added were less well developed than those grown in Pfeffer's solution with thyroxin lacking. Blue Bantam peas were soaked in water for 24 hours and then placed in glass germinators. When the roots were well started the seedlings were placed in 500-cc. jars filled in the first experiment with Pfeffer's solution and in the second with water. In each case 1 mg. sodium salt of thyroxin

TABLE IV

Pisum sativum grown in water with thyroxin added

	Treated, 50 plants	Control, 50 plants	Percentage difference in treated plants
Green tops			%
Total height (cm.)			
After 12 days	327.60	316.60	+ 3.47*
After 26 days	624.00	619.50	+ 0.72
Average height (cm.)			
After 12 days	6.53	6.33	+ 3.25
After 26 days	12.48	12.39	+ 0.72
Total green weight (gm.)	41.00	36.25	+13.10
Average green weight (gm.)	0.82	0.73	+13.10
Roots			
Total length (cm.)	563.00	762.00	−12.99
Average length (cm.)	11.26	15.24	−26.11
Total green weight (gm.)	28.40	27.00	+ 5.18
Average green weight (gm.)	0.57	0.54	+ 5.18

* Plus sign indicates that the treated showed greater growth than the controls, the minus sign that they exhibited less growth.

was added to half of the jars. The results of the experiment in which Pfeffer's solution was used are not fully recorded here, for a slimy bacterial film collected about the roots. In general the controls showed a somewhat greater growth. The results of the experiment in which water was used are recorded in table IV. Here the treated show the greater growth and greater green weight. The length of the primary root of the treated was shorter, as shown in table IV. The roots of the treated were slightly thicker, less branched, and heavier than the controls in spite of the stunting in length. However, the presence of a film on the roots of the treated material may have added slightly to the weight, although the roots were thoroughly washed and dried.

The experiments with peas showed as a whole that thyroxin had no noticeable influence on the length of the tops, but did cause a shortening of the roots. The treated plants exhibited increased weight but probably not enough to be significant.

Discussion

The investigation of the influence of ductless-gland secretions on the growth of plants has not drawn many workers. Those who have experimented in this field have grown plants in a nutrient solution or in water to which the thyroid material was added. Such a plan can hardly be satisfactory, since it is possible that the thyroid material may react with substances in solution, or that bacterial action in a solution containing thyroxin may influence growth. The present study for the most part made use of the injection method in order to avoid these possible difficulties.

The time of flowering of *Allium* and *Narcissus* was accelerated by the presence of thyroxin. At one time in the development of *Allium* there were three times as many open inflorescences among the treated plants as among the controls. Treated *Allium* plants had about 23 per cent. more inflorescences open, and 16 per cent. more flower stalks 102 days after planting. The flower stalks of the treated bulbs of *Allium* averaged at least 50 per cent. taller. There was not much difference in the time of opening of the inflorescences of the treated and untreated bulbs of *Narcissus,* but there was a three-day interval from the opening of the first inflorescence of the treated material before any controls blossomed. This acceleration is to some degree substantiated by the results of REBELLO (**5**), who found that the white bulbs of hyacinth were accelerated in growth; and in principle is affirmed by the results of NIETHAMMER (**4**), who found that resting buds were activated by thyroxin.

The roots of *Allium* and *Pisum* were stunted in the thyroxin solutions. The method of treating the plants was different in the two cases but the results were much the same. The weight of the roots of the treated bulbs

of *Allium* was at least 20 per cent. less than that of the controls. The extra weight may have been due partly to the film which developed on the roots. The stunting effect agrees with the results obtained by BUDDINGTON, who found that the root tips of *Allium* were retarded in growth by thyroxin.

The majority of observations indicate that thyroxin has a somewhat depressing effect on green vegetative growth of *Allium, Narcissus,* and *Pisum sativum* but the results were variable at different dates and with different plants. *Narcissus* showed an acceleration in growth of the treated at every date of observation; *Vicia faba* showed a depression in 75 per cent. of the observations; *Pisum* grown in Pffeffer's solution containing thyroxin was depressed in 88 per cent. of the cases.

Summary

1. *Allium* and *Narcissus* bulbs and *Vicia faba* plants were treated with injections of thyroid material, while *Pisum sativum* seedlings were grown in solutions containing thyroxin.

2. The time of flowering of *Allium* and *Narcissus* was hastened in the thyroxin-treated plants. *Allium* plants showed a stimulation of at least 16 per cent. in time of flowering and in height and number of flower stalks. Acceleration in the flowering time of *Narcissus* occurred also but was not so marked.

3. Roots of treated *Allium* plants were lighter in weight by 20 per cent. than the controls. Roots of the *Pisum* material were shorter by 26 per cent. than those of the controls but showed greater weight, possibly because of adhering bacterial film.

4. Green vegetative parts in general were depressed in growth among the treated plants, although they sometimes showed an early temporary acceleration, especially *Narcissus* and *Pisum.*

These experiments were carried out in the greenhouse of the University of Colorado under the supervision of Professor EDNA L. JOHNSON; to her and to Professor FRANCIS RAMALEY the writer is indebted for valuable suggestions in the experimental work and in the preparation of the manuscript.

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BRIEF PAPERS

EFFECT OF HIGH FREQUENCY SOUND WAVES ON OXIDASE ACTIVITY

(WITH ONE FIGURE)

During the last few years numerous papers have appeared dealing with the chemical and biological effects of high frequency sound radiation. In the present investigation its effects on the activity of oxidase have been determined.

The apparatus producing high frequency sound radiation was similar to that used by HARVEY, HARVEY, and LOOMIS (**2**). The waves were produced by a quartz crystal measuring 2.5 cm. square and ground to give a natural frequency of 450,000 cycles. It was immersed in an oil bath, supplied with an adequate cooling system. This crystal was energized by means of a step-up air transformer, the primary of which was the tank coil of an oscillating Hartley circuit. The plate of the 75-watt tube was supplied with a 60-cycle unrectified current at a voltage of 3500 (r.m.s.).

Fruit extracts were prepared from apricots, peaches, and avocados. The fruit was frozen, ground, dispersed in a large volume of water, and filtered. The oxidase activity of these extracts as measured by the rate of oxygen absorption in the presence of catechol was similar to that of oxidase preparations obtained by the alcohol precipitation method.

Five cc. of the extract were introduced into a thin-walled bulb, which was suspended in the oil bath so that its bottom was about 5 mm. above the crystal. Samples were withdrawn from time to time and their oxidase activity determined by means of a WARBURG respiration apparatus (**3**). The rate of oxygen absorption at 25° C. was measured, using an acetate buffer of pH 4.9 in the presence of 1.5 per cent. of catechol as substrate.

In all cases exposure to the sound waves resulted in a considerable decrease of oxidase activity. No complete inactivation was obtained in treatments up to 12 hours. When the time of exposure was plotted against oxidase activity, all curves approached a logarithmic form; however, the data were not strictly reproducible with the equipment used. A typical curve is shown in figure 1.

In order to correct for effect of temperature, controls were incubated at 38° C., the highest temperature observed in the exposed tube. As shown in the figure, the decrease in activity at this temperature was negligible in comparison with that produced by high frequency sound waves.

The action of sound waves has been frequently ascribed to oxidative processes. It was found, however, that washing out most of the air with

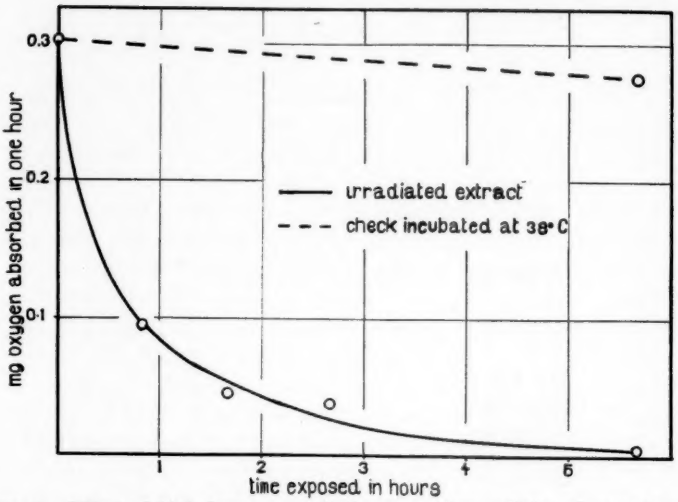

FIG. 1. Effect of high frequency radiation on oxidase activity of avocado extract under nitrogen.

hydrogen or nitrogen by bubbling the gas through the solution before treatment, and also by continuing the washing during the treatment, produced no significant differences in the effect of irradiation.

Since recently BEUTHE (1) has attributed the effect of high frequency sound waves to the formation of hydrogen peroxide, the amount of H_2O_2 formed during irradiation of distilled water was measured by iodine titration. When 100 times this concentration of H_2O_2 was added to the fruit extract, its oxidase activity was not affected appreciably.

It is seen that high frequency sound waves progressively destroy oxidase activity. It would appear that oxidation played a minor rôle in this destruction.—RALPH J. CHRISTENSEN and RUDOLF SAMISCH, *Fruit Products Laboratory, University of California.*

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LATERAL WATER TRANSFER IN LEAVES OF *GINKGO BILOBA*

(WITH ONE FIGURE)

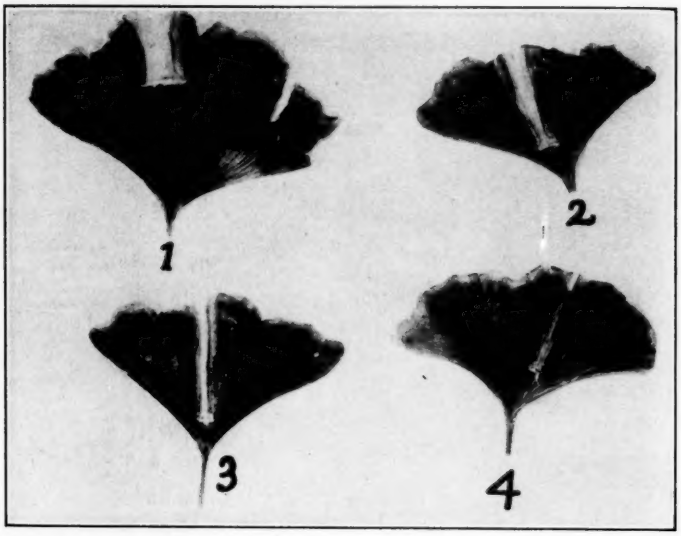
Some years ago, while the writer was investigating the mass factor[1] in the energy relations of leaves, an interesting observation was made on the lateral transfer of water in the leaf tissues of *Ginkgo biloba*. When the leaves of *Ginkgo,* still attached to the twig, had been perforated with the Ganong leaf punch, it was noticed that the tissues distal to the cut died from desiccation within 24 to 48 hours. The edges of the dead tissue ran parallel to the venation of the leaf, but a certain amount of lateral transfer of water from uncut veins into the region whose water supply had been cut off was observed. At that time it was not possible to follow up this interesting problem.

Punching the leaves of ordinary dicotyledonous net-veined leaves does not greatly hamper water distribution throughout the leaf. Leaflets of the bush honeysuckle (*Lonicera morrowi*), for instance, have been cut transversely near the base of the leaflet in such a manner as to sever the midvein water supply, and to leave less than a millimeter of uncut tissue on each flank of the leaflet. Such cut leaflets, if supported mechanically so that they are not torn by air movement, remain green and turgid for days, showing that water enough for the entire leaf can be distributed to the tissues beyond the cut, through the very small uncut basal marginal vein system. It should be extremely interesting to observe under a microscope the distribution of dyes in leaves thus injured.

Ginkgo, with its dichotomous, parallel venation, and with no connection between the veins except mesophyll cells, presents an entirely different problem when the veins are severed transversely. In such a case the transfer of water into the tissues distal to the severed region must be accomplished by a lateral diffusion of water from the nearest uncut veins. To determine how far water can be transmitted laterally through the mesophyll cells with speed sufficient to maintain life, leaves were cut during the seasons 1931, 1932, and 1933, and some measurements made.

In figure 1 are shown some of the leaves after they had developed the necrotic areas after transverse cutting of the veins. In leaf no. 1 the cut is about 10 mm. long; in no. 2, 5 mm.; no. 3, 4 mm.; and no. 4, 2.5 mm. Leaves cut to a less degree than no. 4 did not show death of tissues beyond the severed region. As may be clearly observed in the photograph, the water travels laterally and maintains the life of the tissues to a somewhat variable distance. Careful measurements from the first uncut vein on

[1] SHULL, CHARLES A. The mass factor in the energy relations of leaves. Plant Physiol. 5: 279–282. 1930.



either side to the edge of the tissue which is killed show that the lateral diffusion of water is rapid enough to supply the cells in certain cases to a distance of 2.6 to 3 mm. In traversing this distance the water passes through three or four interveinal tracts, as the vascular bundles are usually less than 1 mm. apart. In leaf no. 4 the dead tissue at the narrowest point seems to represent just one interveinal tract. In this instance the lateral transfer is effective through a shorter distance than in leaf no. 1. The leaves vary considerably in their ability to transmit the water through the mesophyll cells.

The results seem to depend somewhat on the age and condition of the leaf at the time of cutting, and its position on the tree. In more exposed situations the tissues die to a greater extent than in less exposed situations. Young leaves seem to transfer water laterally farther than old leaves. In some cases young leaves seemed to become adjusted, and transmitted the water farther than would have been the case if the leaves had been cut after a greater degree of maturity had been attained. Possibly the cells retain thinner walls, and maintain a more permeable protoplasm in this region, when the cut is made early. To establish this point would require a careful investigation of the aging of leaf tissues; and a comparative study of protoplasmic permeability in young and old leaves, and in old regions which had been isolated by cutting while young. No such studies have been made.

The distance to which water could be transported laterally and successfully maintain cell turgor was much less than had been anticipated. The experiment is one that lends itself very readily to elementary class instruction, and requires only a *Ginkgo* tree, a pen knife, and a millimeter rule. The contrasting behavior of net-veined leaves is easily established by similar methods.—CHARLES A. SHULL, *University of Chicago.*

COMPARISON OF ANATOMICAL AND HISTOLOGICAL DIFFERENCES BETWEEN ROOTS OF BARLEY GROWN IN AERATED AND IN NON-AERATED CULTURE SOLUTIONS

It has been observed by workers on absorption problems in this laboratory that the roots of barley grown in an aerated culture solution have a strikingly different growth habit from those grown under non-aerated conditions. The primary roots of the former are several times as long and the secondaries less numerous. This paper is a preliminary report of a study undertaken to ascertain whether there were any well defined anatomical or histological differences accompanying these different types of root systems.

Two culture tanks made of black sheet iron coated inside with a non-toxic asphaltum paint were used, each tank having a capacity of 112.4 liters. Hoagland's culture solution supplied the necessary inorganic salts, the original volume in each tank being maintained by the addition of distilled water to replace the loss due to transpiration. In one tank aeration was effected by means of fine continuous streams of washed air from an air compressor; in the other the oxygen supply was limited to that diffusing downward from the surface of the culture solution.

A pure strain of barley of the Sacramento variety was employed, a great number of seeds being germinated in the usual manner, and from these the necessary number of seedlings selected for the experiment. Selection was made on the basis of uniformity of size, number of leaves, length of leaves, etc. Thirty-two such plants were grown in each tank.

Once every week for a period of two months two plants were taken from each culture tank. The leaves were counted and the length of each leaf measured. The whole root system was cut off and floated in a large tank of water. With care the roots could be separated, counted, and their lengths measured. The number of roots refers to those emerging from the stem plate or crown. At each sampling transverse sections for microscopic examination were prepared from at least ten different roots taken from the plants grown in the aerated culture solution, and a like number from those grown in the non-aerated solution. The sections were taken from the following positions back from the root tip: 5, 15, 25, 35, 45, 55 mm., and at

the top of the roots. They were stained and made up into permanent slides. A careful study was made of the prepared slides and the following method of examination was adopted. The diameter of the roots was measured, as was also the thickness of the cell walls of the cortex, endodermis, pericycle, xylem vessels, and the large central vessels. The measurements were all made with oil immersion.

The results of the macroscopic examination may be summarized as follows:

1. During the first month all of the plants had about the same number of roots. After this period the number of roots produced by the non-aerated plants increased very rapidly, whereas those of the aerated increased but slightly, and at the end of sixty days the plants growing in the non-aerated culture solution had an average of 225 roots and those grown in the aerated culture solution an average of 75 roots.

2. Differences in root length became evident within a few days after planting, and at the end of sixty days the average length of the roots in the aerated solution was 37.4 cm. as against 10.9 cm. for the non-aerated plants.

3. The roots of the non-aerated plants were about 15 per cent. greater in diameter throughout their entire length than were those of the aerated plants.

4. In general the tops seem to show the same tendencies which were so evident in the root system, *i.e.*, the shoots of the non-aerated plants consisted of a somewhat greater number of shorter leaves than did those of the aerated plants. But the shoot is not influenced by root aeration to the same extent as is the root itself; in fact, individual differences between the tops of plants grown in the same culture solution may be as great as those of plants grown in the different culture solutions.

No weights were taken of the plants described in this paper, but other workers in this laboratory have shown that there is no significant difference in the weights of either the tops or roots of barley plants grown in aerated and non-aerated culture solutions when aeration is effected as described here.

The results of the microscopic examination may be briefly summarized as follows:

1. The cortex of the roots grown in the aerated culture solution consists of uniformly compact parenchyma with no conspicuous intercellular spaces. The cortical region of the roots grown in the non-aerated solution is composed of large air passages separated by narrow strands of parenchyma.

2. The first tissue to differentiate is the xylem vessels of the non-aerated roots. These vessels develop secondary thickening of their walls at a distance of 5 mm. from the root tips. The next is the xylem vessels of the aerated roots at a distance of 15 mm. from the root tips. This is followed

by the pericycle of the non-aerated roots at a distance of 35 mm. from the root tips. At a distance of 45 mm. from the root tips the cell walls of the pericycle of the aerated roots have begun to thicken, as have also those of the central ducts of the non-aerated roots. At a distance of 55 mm. from the tips the cell walls of the endodermis of both the aerated and non-aerated roots have begun to thicken. At the top of the roots next to the crown all of the walls included in the measurements have become secondarily thickened, and the thickness of the cell walls of the roots grown in the aerated culture solution is about twice as great as that of those grown in the non-aerated solution.

It has been shown by others in this laboratory that the concentration of reducing and total sugars of barley roots grown in an aerated culture solution is less than that of those grown in a non-aerated solution. It seems reasonable to assume that the greater amount of oxygen available to the aerated roots, with its consequent accelerating effect on the respiratory rate, decreases the sugar concentration to a point where it becomes the limiting factor in cell wall thickening, particularly so in the lower root fraction. The cell walls of the root near the crown are not so limited, since sugar depletion has not yet taken place to any great extent. Here the greater thickening may reflect a higher level of protoplasmic activity due to a greater oxygen as well as to an adequate sugar supply.

The greater length of the aerated roots may also be accounted for by the greater supply of oxygen available to their apical meristems.

Very briefly, then, the tissues in the roots grown in the non-aerated culture solution start to differentiate nearer the root tip than do those grown in the aerated culture solution. But, starting at about 25 mm. from the root tips, the cell walls of the roots grown in the aerated culture solution start to thicken more rapidly than do those of the plants grown in the non-aerated culture solution, and in the mature regions of the roots these walls are about twice as thick as the corresponding cell walls of the roots grown in the non-aerated solution. These structural and sugar content differences probably have their explanation in the differences in amounts of oxygen in the two culture solutions.

The writer wishes to acknowledge his indebtedness to Professor A. R. DAVIS for the valuable advice received during the course of this work.—A. E. BRYANT, *Laboratories of Plant Nutrition and the Department of Botany, University of California.*

TOLERANCE OF LIQUID-AIR TEMPERATURES BY SEEDS OF HIGHER PLANTS FOR SIXTY DAYS

In 1897 BROWN and ESCOMBE[1] published results of experiments which proved that seeds of a number of different higher plants were uninjured by exposure to liquid-air temperature – 189° to – 193° C.) for 110 hours. Later THISELTON-DYER[2] and BECQUEREL[3] published confirmatory evidence to the same effect and in addition THISELTON-DYER demonstrated that several different kinds of seeds tolerated, without visible injury, the temperature of liquid hydrogen (– 250° C.) for one to six hours. It occurred to us that it would be interesting to learn whether or not seeds can tolerate liquid-air temperatures for much longer periods than 110 hours. Accordingly, we selected nineteen varieties of seeds of higher plants, dried them for one week over calcium chloride, and distributed them into three glass tubes which were immersed in liquid air. One tube was removed from the liquid air after thirty days and the other two tubes after sixty days. After removal from the liquid air the tubes were opened and the seeds tested for germinating capacity alongside of control seeds from the same original lot which had not been subjected to liquid-air temperature. Within twenty-four hours after the seeds were placed between layers of wet filter paper many were germinating, and equally well in the treated and in the control specimens. After a few days practically all seeds of most of the species represented had germinated into vigorous seedlings. Two or three species showed poor germinating capacity, but this was true alike for the control and the treated seeds of those varieties. In order to determine whether or not injury was sustained by seeds at liquid-air temperature which was not apparent during the germination period, seedlings from the seeds tolerating the sixty-day exposure to liquid air are being grown in culture solutions alongside of control seedlings of the same varieties. These have now (30th January, 1934) been growing for about ten weeks in a greenhouse, and there is not the slightest evidence of injury by the liquid air treatment for so long a period as sixty days. In some instances the seedlings from the treated lot of seed are somewhat superior to the controls in amount of growth attained and in other cases the reverse is true, but no significant differences are discernible.

The kinds of seeds treated for thirty days are as follows:

[1] BROWN, H. T., and ESCOMBE, F. Note on the influence of very low temperatures on the germinative power of seeds. Proc. Roy. Soc. London **62**: 160–165. 1897.

[2] THISELTON-DYER, SIR W. On the influence of liquid hydrogen on the germinative power of seeds. Proc. Roy. Soc. London **65**: 361–368. 1899.

[3] Becquerel, Paul. Recherches sur la vie latente des graines. Ann. Sci. Nat. Series 9, **5**: 222–228. 1907.

Early amber sugar-cane	Kenota oats
Stenning spinach	Silver-skinned white Portugal onions
Early white spine cucumbers	Milo maize
Sugar beets	*Melilotus indica*
Japanese buckwheat	Yellow mustard
Sacramento barley	Santa Clara tomatoes
Purple vetch	

In the sixty-day series all of the foregoing seeds were tested and in addition the following:

Canadian peas	Hubbard squash
Golden bantam corn	Grimm alfalfa
American Wonder peas	Russian mammoth sunflower

Many interesting questions arise in one's mind on being confronted by such results as those above described. While space in this paper will not permit a discussion of them all and since we intend to discuss these problems later, mention may be made of only two or three aspects of such an inquiry. Inasmuch as seeds may be immersed in liquid air for sixty days without injury, as we have shown, one might ask the question as to whether or not it would be possible for the life in seeds to be preserved forever at liquid-air temperatures. On the basis of what we know today regarding the longevity of seeds under other conditions than liquid-air temperatures, we are inclined to answer this question in the negative, because no studies have yet been made of any seed, with the exception of *Nelumbium*, which has been shown to have life in it for as great a period as a century. In the experiments with *Nelumbium* it will be recalled that a seed at the British Museum 150 years old was made to germinate and that OHGA found *Nelumbium* seeds which he believed to be several centuries old, all of which were found to have germinating capacity. This leaves the question open as to whether or not some seeds, even though it may not be true of all, may have power to resist deterioration indefinitely. It must also be said, however, that there is another side to this problem, namely, that since most seeds which we know today have a relatively short life, the destruction of enzymes and similar substances or agencies in the seed may lead to the abbreviation of life in seeds quite apart from the question of whether or not the embryo retains its respiratory powers. If, however, it should prove to be the case that at liquid-air temperatures respiration is either extremely slow or nonexistent, and further that the enzymes and other substances to which I have referred, or other processes concerned with the living seed, remain unchanged within the seed, then keeping seeds at such temperatures would indeed be a way of preserving them forever. Further research may perhaps yield some results which will lead to a more definite answer to this problem.

Another aspect of the subject from which our results emanate naturally comes to mind now, namely, the condition of the water in the seeds which are exposed to liquid-air temperatures. Obviously it is water which does not freeze, for if it did freeze it is in the highest degree likely that the seeds would be injured. In other words, therefore, such seeds as we have used with approximately ten per cent of water in them contain the water in the form commonly spoken of as "bound water." To be sure, the term "bound water" is differently defined by different investigators. Nevertheless, we may regard bound water in this case as water which is contained in spaces that are so minute as to render impossible the behavior of that water in the way in which water in large spaces does behave at low temperatures. Just as it is in the highest degree likely that water will not freeze in extremely fine capillary tubes, so water contained in the very minute spaces or interstices of the colloids making up the embryo and the balance of the seed would also be proof against freezing at any temperature, no matter how low.

We are indebted to Dr. G. R. MACDONALD for keeping the supply of liquid air replenished during the experiment and to Mr. W. C. CHANDLER for assistance in the germination tests and in the subsequent culture experiments.—CHAS. B. LIPMAN and G. N. LEWIS, *University of California.*

NOTES

Summer Meeting.—A summer meeting of the Society in cooperation with other botanical groups will be held at Berkeley, California, in connection with the summer meeting of the A. A. A. S. Professor J. P. BENNETT, of the University of California, has been appointed to take charge of all local arrangements in connection with this meeting. It affords opportunity for the western members to get together, and a large attendance is expected. The general headquarters will be at the Stephens Union Building. There will be appropriate symposia and joint meetings with the Botanical Society of America and Section G of the A. A. A. S. The outlook is for an unusually good meeting, and abundant opportunity will be provided for visits to the Bay cities, Mount Tamalpais, and the surrounding country. Stanford University and Davis are not too far away for a visit during the period of the meetings, June 18–23, 1934.

Pittsburgh Meeting.—The eleventh annual meeting of the American Society of Plant Physiologists will be held at Pittsburgh in December, 1934. The local representative is Dr. O. E. JENNINGS, of the University of Pittsburgh. It is not too early to begin planning for the meeting. Situated between east and west, it should not be difficult to set attendance records, and to find a wealth of material from which to select for the programs.

Minnesota Section.—The Minnesota Section has several meetings planned for the spring months. The titles of the papers to be presented and the speakers are as follows:

April 4, 1934, Physiology and anatomy of the embryo sac. F. E. BUTTERS.

May 9, Mineral nutrition. FREDERICK CHANDLER.

June 6, Physiological effects of alcohol. CHARLES ROGERS.

The meetings are always open to those who are not members of the Society, and interested non-members are cordially invited to attend the meetings.

New England Section.—The New England Section is starting its work by sponsoring a meeting at Amherst, Massachusetts, on May 25–26, 1934, from Friday noon to Saturday noon. It is the desire of the local committee that all plant physiologists in this region should attend, and an invitation is extended to all persons interested in botany, plant pathology, horticulture, forestry, agronomy, etc., to meet with them. Anyone is privileged to present a paper. It is hoped that through this sponsorship the plant physiologists and their friends may join in a period of good fellowship, and in

the enjoyment of the interesting programs being arranged for this meeting. Titles should be sent to LINUS H. JONES, secretary of the section, Clark Hall, Massachusetts State College, Amherst, Mass.

Purdue Section.—The Purdue Section reports an interesting series of meetings for 1933–1934. Twelve meetings were arranged for the year with the following programs:

October 16, 1933, The caustic and stimulative effects of x-rays on plants. C. A. SHULL, guest speaker.

November 3, MITSCHERLICH's quantitative method of using plants to determine plant nutrient deficiencies of soils. S. R. MILES.

November 20, The biology of mixed cultures of microorganisms. C. L. PORTER.

December 4, Some effects of soft x-rays on seedlings. H. M. BENEDICT.

January 15, 1934, Reports on the Boston meetings, A. A. A. S.

February 5, Movies taken in Honduras. H. E. ENDERS.

February 19, The chemistry and physiological importance of carotene and related pigments. C. L. SHREWSBURY.

March 5, Root growth habits in relation to performance of inbred and hybrid corn. R. R. ST. JOHN.

March 19, Fruit setting as a physiological problem. LAURENZ GREENE.

April 2, Effect of supplementary artificial radiation on plant growth. R. B. WITHROW.

April 19, Effect of light on the carbohydrate and nitrogen metabolism of asters. RAYMOND WENGER.

May 7, Guest speaker, unannounced.

The attendance at the meetings has varied from 20 to 40, with an average of about 25. The meetings are informal, with an attractive combination of social and scientific interest. During the year G. N. HOFFER has been president of the section, R. B. WITHROW secretary-treasurer, and RALPH M. CALDWELL chairman of the program committee.

Program Committee.—The program committee has been appointed by the president, Dr. C. O. APPLEMAN of Maryland. The members on the committee are as follows: Dr. H. C. SAMPSON, Ohio State University; Dr. P. D. STRAUSBAUGH, West Virginia University; Dr. WARREN B. MACK, Pennsylvania State College; and Dr. H. R. KRAYBILL, Purdue University, chairman. Dr. A. E. MURNEEK, University of Missouri, is *ex officio* member of the committee. Cooperation of all members of the Society in any matters pertaining to the meeting will be greatly appreciated.

Stephen Hales Prize Committee.—The fourth award of the STEPHEN HALES prize will be made at the Pittsburgh meeting. The committee of award according to the by-laws is constituted as follows: Dr. D. R. HOAGLAND, University of California, chairman; Dr. W. W. GARNER, U. S. Department of Agriculture; and Dr. H. B. VICKERY, Connecticut Agricultural Experiment Station. These three members, recipients of the award in past years, have the unique privilege of selecting the fourth investigator to be honored by the Society in this manner.

Chemical Methods Committee.—The personnel of the Chemical Methods committee has been modified by the appointment of Dr. Z. I. KERTESZ of the New York Agricultural Experiment Station (Geneva) as a member of the committee. This committee is engaged in preparation of a supplementary report on chemical methods.

Recent Advances in Plant Physiology.—A second edition of E. C. BARTON-WRIGHT's summary of the recent advances in plant physiology is now available. It is published by P. Blakiston's Son and Co., Philadelphia. There are some changes in organization from the first edition. The discussion of soil is omitted, and the nine chapter headings are: Absorption of water and transpiration; carbon assimilation; nitrogen metabolism; the raw materials of plant nutrition; translocation; respiration; growth; light and growth; and accessory growth factors and related problems. The work covers approximately the last 15 years of progress. The price is $4.00 per copy.

Thermodynamics of Plants.—An interesting and valuable monograph (no. 30 of the Monographien aus dem Gesamtgebiet der Physiologie der Pflanzen und der Tiere), Pflanzenthermodynamik, by KURT STERN, has been published by Julius Springer, Berlin. The first half of the monograph deals with the physical basis of plant thermodynamics; the fundamental laws of thermodynamics; phase rules and phase transformations; and the general nature of chemical, electrical, radiant, and surface energy. The second half of the volume is devoted to applications of these principles to the physiological processes of plants. This is the most useful portion of the book, but the general principles must be thoroughly mastered before the applications can be appreciated. The applicability of the first and second laws; the thermodynamics of phase transformations; and the thermodynamics of the chemical and electrical processes of plant cells are covered. There is a special chapter on thermodynamics of carbon assimilation, and one on surface phenomena. The final chapter is a general summary. Those who are interested will find a wealth of information brought together

in illuminating fashion. The price of the work in brochure binding is RM 32, bound RM 33.2 per copy.

Progress of Botany.—The second volume of the Fortschritte der Botanik which is edited by FRITZ VON WETTSTEIN, München, was published late in 1933 by Julius Springer, Berlin. It covers the main advances for the year 1932. The fields summarized are morphology; systematics, paleobotany, and genetic plant geography; physiology of metabolism; physiology of growth, heredity, and development; and a brief supplement devoted to ecology. There are sixteen collaborators responsible for the seventeen individual sections. This annual is a valuable work for both teacher and investigator. It assists one in maintaining a bird's-eye view of the progress of botany, and its current trends. The quoted price is RM 24 for brochure binding.

Researches on Fungi.—Volume V of A. H. REGINALD BULLER'S work, Researches on Fungi, was published late in 1933 by Longmans, Green and Co. Among other things the author gives an account of the translocation of protoplasm through the septate mycelia of higher fungi and the causes of such translocation. He thinks that the new facts brought to light in this work may have bearing upon the problem of translocation of organic substances through the sieve tubes of the higher plants. Physiologists will find many interesting observations in the volume. The price of Volume V is 26 shillings.